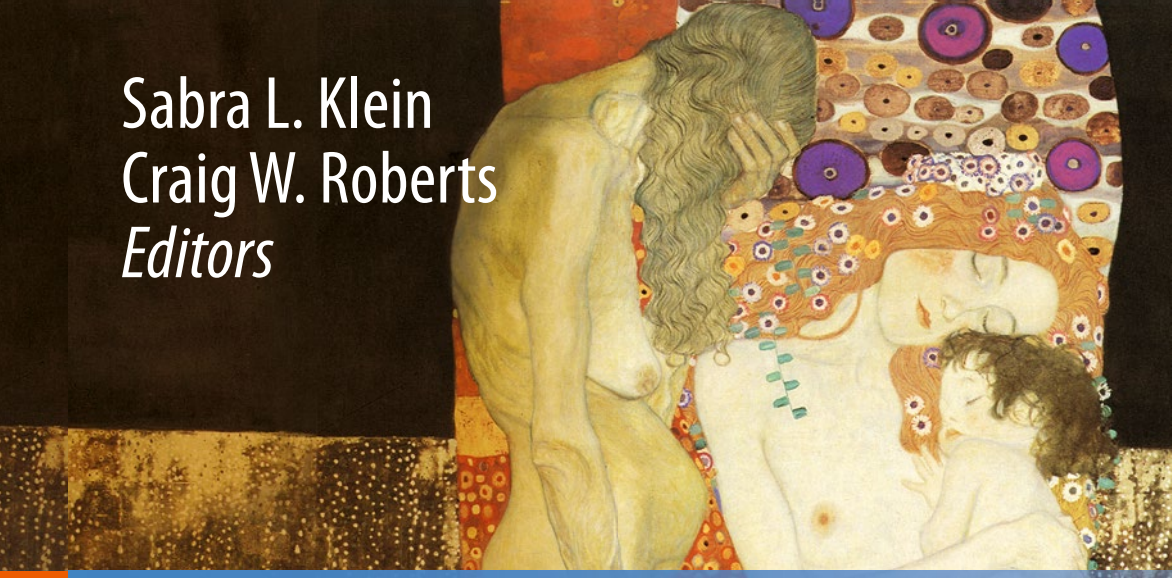
Sabra L. Klein
Craig W. Roberts
Editors



Sex and Gender Differences in Infection and Treatments for Infectious Diseases

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Editors
Sabra L. Klein Ph.D
Department of Molecular
Microbiology & Immunology
Johns Hopkins Bloomberg
School of Public Health
Baltimore
Maryland
USA

Craig W. Roberts Ph.D
Professor of Parasitology
Strathclyde Institute of Pharmacy &
Biomedical Sciences
University of Strathclyde
Glasgow
United Kingdom

ISBN 978-3-319-16437-3 ISBN 978-3-319-16438-0 (eBook)
DOI 10.1007/978-3-319-16438-0

Library of Congress Control Number: 2015943677

Springer Cham Heidelberg New York Dordrecht London

Cover Picture Credit: Directorship of the National Gallery of Modern and Contemporary Art of Rome. Courtesy of the Ministry of Cultural Heritage and Activities.

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Foreword

In 1988 I was invited, as an immunologist working on parasites, by the then Editor of *Parasitology Today* to debate with the epidemiologist, Don Bundy, the reasons why males and females often display different incidences, prevalence, and severities of certain parasite infections. The perceived wisdom at the time, although acknowledging that physiological factors may play a role in these observations, suggested that clear gender-related differences as a result of sociocultural differences in behavior between the sexes were primarily involved. However, the general consensus derived from the debate would suggest, as a general rule of thumb, that while the prevalence and incidence of infection could be largely attributed to relative gender differences in behavior leading to differential exposure, the comparative severity of disease was a result of sex biased immune responses. At the time of the debate, while there had been numerous observations of age and sex-determined patterns of infection little had been done to determine the functional basis of these observations. Estrogen receptors, for example, were only demonstrated for the first time in macrophages in 1990 by my coauthor on the *Parasitology Today* article, Bill Stimson.

I would like to think that the debate in 1988 initiated significant interest within the research community as to the potential of hormones to influence immune activity and determine the outcome of infectious disease. Indeed it certainly inspired a young graduate student in my lab at the time, Craig Roberts, who insisted as part of his doctoral studies he compare the course of infection and immune responses in male and female mice infected with *Toxoplasma gondii*. Little emphasis had been placed by workers in the field on sex differences at that time, and indeed experimental and control groups in immunological studies often comprised mixed groups of males and females despite the fact that sex differences in laboratory models had been noted, but in large part ignored, some 10 years previously. It reminded me of a criticism of our field of study by Hauschka in 1947 that was perhaps equally pertinent in 1988; “while critical attention has been paid to species, genetic strain, age, weight, and diet of experimental

hosts, sex as an environmental factor has been treated with comparative neglect throughout the literature in protozoan parasitology." Craig demonstrated that female mice were more susceptible than male mice to infection with *T. gondii*, and this was related both to the differential kinetics as well as the magnitude of the immune response between the sexes. Interestingly, and probably as a result of these observations, the Roberts lab is generally now asked by reviewers to provide information on both sexes when submitting manuscripts on *T. gondii* infectivity for publication. I have always agreed that this is a perfectly valid request and if feasible should always be complied with. Consequently, it is of major significance that the National Institutes of Health (NIH) in the United States recognizes the importance of gender and sex-mediated effects and is formulating guidelines that will require in all future applications that balance and equality between male and female cells and animals are maintained in all preclinical studies except in exceptional circumstances. From my experience with regulatory authorities at local and national levels, the severe morbidity and high mortality rates following *T. gondii* infection in the females of some mouse strains have significantly limited their use for ethical reasons. It could, with some justification, be argued that this is an exceptional circumstance and for ethical reasons females therefore be excluded from studies. However, that acknowledged, their use in vaccine or therapeutic studies should not be excluded as they also provide a potential "gold standard" for therapeutic efficacy. The NIH directive will certainly focus minds as to appropriate and ethical gender-related experimentation and promote good research.

Since 1988, tremendous progress has been made in determining how hormones influence immune cell function. Hormone receptors within immune cells have been characterized, and the mechanisms by which these influence infectious and noninfectious diseases have been scrutinized. Sabra Klein and Craig Roberts edited their first book in 2010, "Sex hormones and immunity to infection," which summarized much of the progress that had been made to that date in a single volume. Notably, most of this book dealt with the evolution of these differences, potential reasons why these differences exist, and the mechanisms responsible, as well as highlighting in which diseases their influences are most notably expressed. The time is now appropriate and the knowledge base sufficient to begin to translate these findings into finely tailored gender/sex-specific therapies. The era of sex-specific medicine, if not truly personalized medicine, has begun and should benefit both males and females equally.

The second book jointly edited by Klein and Roberts not only updates and complements the previous tome but also extensively widens its remit to cover potential practical applications of new and acknowledged gender and sex-related influences. It comprises a series of up-to-date reviews by experts in their respective fields that comprehensively covers the area of sex and gender differences and control of infectious diseases. The book is well structured consisting of two sections: the first underpins the genetic and physiological basis of sex differences

and immunity and the second consists of a series of reviews that deal with how these differences relate to specific diseases or groups of diseases. Given the new directive coming from the NIH that gender and sex equality is imperative in preclinical trials, this book is not only timely but should also be essential reading for all biomedical scientists.

University of Strathclyde
Glasgow, UK

James Alexander

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Chapter 1
Sex Differences in the Immune Response

Carole L. Galligan and Eleanor N. Fish

Abstract The distinct differences between males and females in the incidence of infections, the severity of disease, and the likely outcome are a consequence of sex-related differences in immune cell composition and activation following exposure to a pathogen. Here, we review the effects of age, hormones, and genes on shaping an immune response and how this affects disease pathogenesis differently for males than females. Viewed altogether, the sex-dependent effects on the immunophenotype should be considered for the optimum implementation of effective therapeutic interventions, whether these be related to treatment of pathogenic infections or related to prevention, as in the case of vaccination.

1.1 Introduction

The role of sex differences in an immune response continues to receive little attention. There is accumulating evidence that sex differences have profound effects on the immune system, and the failure to take these into account, either by blending data from both males and females or, worse yet, taking scientific findings made in one sex and applying these to both sexes, will lead to erroneous conclusions (Stanberry et al. 2002). There are inherent differences in the susceptibility of males and females to a variety of different pathogens and to different autoimmune diseases. This suggests fundamental differences in the immune system—the immunophenotype—of males and females. These differences are multifactorial and include differences in the number of specific immune cell types and their activation response to immunological challenge following vaccination or exposure to a pathogen. Sex bias might result from differences in hormone levels, might be related to X- or Y-linked genes, or might be a consequence of environmental factors. Here, we review the current literature that supports a role for sex-based differences in the immune response.

C.L. Galligan • E.N. Fish (✉)
Toronto General Research Institute, University Health Network & Department of Immunology, University of Toronto, Toronto, ON, Canada, M5G 2M1
e-mail: en.fish@utoronto.ca

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_1

1.2 Sex Biases in Response to Infection

Notable differences continue to be reported between males and females in response to infection by diverse pathogens. Generally, males are more susceptible and females more resistant to bacterial, viral, and parasitic infections, which is especially prominent between puberty and menopause (Bouman et al. 2005; Dao and Kazin 2007; Napravnik et al. 2002; Klein 2000). Conversely, females have a higher prevalence of autoimmune diseases following puberty and prior to menopause. In humans, accumulating data indicate that males are more likely than females to contract viral infections (Klein et al. 2010a), regardless of viral genotype, e.g., HIV (Farzadegan et al. 1998; Sterling et al. 2001), hepatitis B virus (HBV) (Shimizu et al. 2007), West Nile virus (Jean et al. 2007), influenza (Klein 2012b), and hantaviruses (Williams et al. 1997; Armien et al. 2004). Independent of initial infection rates, disease progression and outcomes also exhibit a sex differential. For example, females with similar HIV viral loads as males have a more rapid progression to AIDS (Farzadegan et al. 1998). Despite equal numbers of cases, infected females of reproductive ages are 2–6 times more likely to die from H5N1 avian influenza than males (Klein 2012a), underscoring a fundamental difference in the pathophysiology of viral infections in males and females.

These differences in disease incidence are not restricted to viral infections: worldwide there is almost a twofold increase in the proportion of adult males with symptomatic *Mycobacterium tuberculosis* when compared to females, but this is not observed in infants or young adults (Neyrolles and Quintana-Murci 2009) (see Chap. 8). Similarly, in mice, females are more resistant to *Mycobacterium intracellulare* and *Mycobacterium marinum* infection (Yamamoto et al. 1990, 1991). Males have a higher incidence of *Helicobacter pylori* (Valliani et al. 2013), *Coxiella burnetii* (Leone et al. 2004), *Pseudomonas aeruginosa* (Sivanmaliappan and Sevanan 2014), and *Salmonella typhimurium* (Afroz et al. 2011) infections. Sepsis is a systemic inflammatory response to infection, most commonly occurring in response to bacterial infection. Women are more likely to survive sepsis than men (Schroder et al. 2000), perhaps due to having a lower initial bacterial burden than men (see Chap. 9).

The male bias in the incidence of infection extends to parasitic and fungal infections. In hypo-endemic regions of Asia, males have a higher incidence of malaria than females (Pathak et al. 2012). Female mice are more resistant than males to infections with *Plasmodium chabaudi* (Wunderlich et al. 1991; Cernetich et al. 2006). Additionally, female mice exhibit a stronger inflammatory reaction to *Schistosoma mansoni* infection than males (Boissier et al. 2003). Differences in behavioral exposures, environmental factors, and cultural factors or a combination may contribute to this male/female bias. Despite not knowing the precise causes, the overwhelming trend for males to be more susceptible than females to infection is suggestive of a fundamental difference in the pathogenesis of disease in males and females. These fundamental differences will be discussed in more detail in the later chapters of this book.

1.3 Sex Differences in the Immune Response

1.3.1 Innate Immunity

In general, females generate stronger innate and adaptive immune responses compared with males (Bouman et al. 2005; Ackerman 2006; Gleicher and Barad 2007; Rubtsov et al. 2010). The innate immune system is critical in protecting the host from the pathogens, since it is the first line of immunological defense. The immune cells of the innate immune response include neutrophils, monocytes/macrophages, dendritic cells, basophils, eosinophils, mast cells, and natural killer (NK) cells that provide nonspecific protection. Monocytes and neutrophils can directly phagocytize bacteria, viruses, and protozoa, which may aid in reducing pathogen load. Additionally, innate immune cells produce oxygen radicals and release enzymes that are cytotoxic and also are capable of processing pathogenic antigens for presentation to naïve T cells to invoke an adaptive immune response. There are several reports that indicate that the profile of innate immune cells differs between males and females. Monocytes normally comprise 5–10 % of the circulating white blood cells. Human males are reported to have higher numbers of monocytes in their circulation compared to females (Bouman et al. 2004). By contrast, female macaques have significantly higher monocyte counts in their peripheral blood compared to males (Xia et al. 2009). Male mice have higher circulating neutrophil counts (Doeing et al. 2003; Peters and Barker 2014) than female mice, although this difference is both age and strain dependent. Female mice have higher numbers of resident cells in the pleural and peritoneal cavities than male mice (Scotland et al. 2011). Male rats have higher numbers of peritoneal mast cells compared to females, with almost twofold higher histamine content in the cell (Jaques and Ruegg 1970).

NK T cells are unconventional T lymphocytes that recognize the non-polymorphic CD1d molecule and recognize foreign glycolipids. While there is some variability in the absolute numbers of circulating NK T cells, human females have significantly higher numbers compared to males (Kee et al. 2012). While these differences have not been consistently reported, this trend toward higher numbers of NK T cells in human females is supported by accumulating evidence (Sandberg et al. 2003; Montoya et al. 2007).

In addition to differences in absolute numbers of immune cells, several reports in humans have indicated that there may be sex differences in the extent of activation of innate immune cells. LPS stimulation of human monocytes from males invokes higher levels of cytokine secretion (e.g., IL-1ß, TNF-α, and IL-12) compared with female-derived monocytes (Bouman et al. 2004). Similarly, neutrophils from human males express more TLR4 and respond to LPS stimulation with greater TNF-α production than those derived from females (Aomatsu et al. 2013). This hyperresponsiveness of male-derived neutrophils to LPS was suggested as a potential mechanism whereby males are more susceptible to sepsis than females (Aomatsu et al. 2013). Human male-derived neutrophils also were more responsive

to IFN-γ stimulation than those from females, suggesting male neutrophil hyperresponsiveness extends beyond TLR4 signaling (Aomatsu et al. 2013). In another study using an airway inflammation model of asthma, there was evidence that ovalbumin-immunized female mice had twice as many macrophages and dendritic cells migrating to draining lymph nodes compared with males, suggesting that females would trigger a stronger adaptive immune response (Melgert et al. 2010). In this same study, female mice exhibited a higher percentage of eosinophils and ovalbumin-specific IgE in their lungs than male mice (Melgert et al. 2010).

1.3.2 Pathogen Detection

Inherent differences in the ability of the immune system of males and females to detect invading pathogens may also contribute to sex differences in the outcome of infection. For example, there is emerging evidence that sex-related differences in HIV-1 detection exist (Meier et al. 2009). Viral detection is mediated by pathogen-associated microbial pattern recognition (PAMP) receptors that include TLRs and cytoplasmic helicases, which detect viral genetic material. Higher levels of the single-stranded RNA microbial pattern recognition receptor, TLR7, have been reported in female compared with male mice (Pisitkun et al. 2006). Indeed, the activation of PBMCs from human females with TLR7 but not TLR9 agonists induced higher levels of IFN-α compared with PBMCs isolated from males (Berghofer et al. 2006). Plasmacytoid dendritic cells (pDCs) are specialized cells that produce high levels of type I IFNs in response to TLR7 and TLR9 activation and exert a critical role in an antiviral response (Gilliet et al. 2008). Human female-derived pDCs generate more IFN-α production in response to HIV-1-induced TLR7 activation compared with those from males (Meier et al. 2009). Whole lung extracts from female Norway rats infected with Seoul virus, a hantavirus, have higher levels of TLR7-, RIG-I-, and IFN-induced gene expression compared with lung extracts from similarly infected males (Hannah et al. 2008). Additionally, peritoneal and pleural macrophages derived from female mice express higher levels of TLR2, TLR3, and TLR4 and demonstrate enhanced phagocytosis and bacterial killing compared with cells derived from males (Scotland et al. 2011). These data suggest that females may be better positioned than males to mount an immune response to specific pathogens, specifically as a consequence of their higher levels of expression of PAMP receptors than males.

1.3.3 Adaptive Immunity

The adaptive immune response involves both cellular and humoral effectors associated with T and B lymphocytes. Sex-specific differences in the number of

circulating human T cells have been reported, with females having higher levels of circulating CD3 lymphocytes than males (Das et al. 2008; Bouman et al. 2004). CD3 lymphocytes can be broadly subdivided into CD4+ and CD8+ T subsets. CD4 T cells are involved in cytokine release, B-cell class switching, and maximizing bactericidal activity of the innate immune system. CD4+ T cells can be subdivided into many effector subsets on the basis of their cytokine secretion. These include IFN-γ- and IL-12-secreting T helper (Th) 1 cells; IL-4-, IL-5-, and IL-13-secreting Th2 cells; IL-17-secreting Th17 cells; and IL-9-secreting Th9 cells. Additionally, CD4+ cells originally defined as expressing the transcription factor, FoxP3, suppress T-cell proliferation, secrete IL-10, and are named T regulatory cells (Tregs). Recently, another subset of CD4+ T cells expressing BCL-6 were identified and named follicular B helper T cells (TFH). TFH are involved in stimulating B-cell-derived antibody production. CD8+ T cells induce lysis of cells infected with intracellular pathogens (including viruses), tumors, or autologously transplanted tissues and are aptly named T cytotoxic cells (Tc). CD8+ cells can also be further subdivided into Tc1, Tc2, and Tc17, based on their cytokine secretion. Tc1 cells secrete IFN-γ, Tc2 cells secrete IL-4, and Tc17 cells secrete IL-17.

There is a dearth of information on sex differences in immune cell subsets. Human females have higher levels of circulating CD4+ T cells and their CD4:CD8 ratios are higher (Das et al. 2008; Amadori et al. 1995). Similarly, female macaques have higher numbers of circulating CD4+ and CD8+ T cells compared with males (Xia et al. 2009). Certainly, an immune challenge can expand T-cell subsets. In mice and humans, T-cell activation in females resulted in increased numbers of CD4+ T cells in the lungs and peripheral blood, respectively (Melgert et al. 2010; Zhang et al. 2012). Additionally, activated human peripheral blood CD4+ T cells from females produced higher levels of the Th1 cytokine IFN-γ when compared with males (Zhang et al. 2012). Similarly in human PBMCs, cytomegalovirus (CMV) challenge of female PBMCs results in greater production of IFN-γ and IL-2 compared with PBMCs from males. Given the association of IL-2 with T-cell expansion, this may contribute to lower T-cell numbers in males (Bouman et al. 2004). A microarray analysis of activated human CD4 and CD8 T cells revealed differential expression patterns in cells derived from females compared to males (Hewagama et al. 2009). Notably, elevated levels of the Th1 cytokine IFN-γ and the cytotoxic T-cell enzyme granzyme A were observed in females (Hewagama et al. 2009). These sex biases in T-cell subset numbers and activity likely contribute to sex differences in infection, immunity, and autoimmunity.

B lymphocytes are primarily antibody-producing cells that comprise 5–15 % of the circulating white blood cells. While human females have higher basal levels of IgG than males (Butterworth et al. 1967), there is little evidence for a difference in B-cell numbers in the circulation of females compared with males. However, female nonhuman primates have higher levels of B cells (Xia et al. 2009), and there is some evidence that human females have higher levels of the activated B-cell subset, defined by the expression of CD23b (Rovati et al. 2013).

1.4 Potential Etiology of Sex Differences in Immune Responses

1.4.1 Hormones

The female prevalence of many autoimmune diseases (Pennell et al. 2012) has suggested a role for hormones in immune cell activation. Indeed, higher levels of 17β-estradiol are reported in patients with rheumatoid arthritis than age-matched healthy controls (Straub et al. 2005), and autoantibodies against the intracellular estrogen receptor alpha (ERα) are present in the serum of patients with systemic lupus erythematosus (SLE). These antibodies behave in a similar manner to the natural ligand and activate the receptor, which may augment the immune response (Colasanti et al. 2012). There is an added complexity associated with fluctuations in hormone levels in females that occur during the menstrual cycle and pregnancy as well as during different stages in their life including pre- and postpuberty and after menopause.

Certainly, estrogens influence the ability of cells to become infected. 17-β-estradiol can regulate the expression of surface receptors mediating viral entry into target cells. This has been observed for HIV-specific chemokine receptors and for αVβ3 integrin, which determines adenovirus, coxsackievirus A9, and hantavirus cell entry (Mo et al. 2005; Wickham et al. 1993; Roivainen et al. 1994; Gavrilovskaya et al. 1998; Woodward et al. 2001). Estrogens also affect the outcome of infections. For example, ovariectomized mice are more likely to become infected with *Coxiella burnetii* than intact control mice, whereas 17-β-estradiol treatment in female mice is protective (Leone et al. 2004). Testosterone treatment of female mice or castrated male mice results in an enhanced rate of infection with *Mycobacterium avium*, whereas 17β-estradiol treatment confers resistance (Tsuyuguchi et al. 2001).

There are two functionally distinct intracellular ERs: ERα and ERβ. Estrogen binding results in ER translocation to the nucleus where the hormone-receptor complex can bind estrogen responsive elements in DNA and regulate gene transcription (Cunningham and Gilkeson 2011). In addition to being expressed in the female reproductive tract, ERs are expressed in many immune cells including the B and T lymphocytes, neutrophils, macrophages, NK cells, thymic stromal cells, bone marrow, and endothelial cells (Bouman et al. 2005; Ackerman 2006; Heldring et al. 2007). There has been some speculation that different ER isoforms and variable estrogen affinity for these contribute to cellular sensitivities to estrogens (Ackerman 2006). A membrane-associated ER, called the G protein-coupled ER (GPER), that modulates signal transduction cascades has been described (Revankar et al. 2005). GPER has been detected in B-cell lymphoblasts (Owman et al. 1996) and a neutrophil cell line (Blesson and Sahlin 2012); however, the functional significance of this receptor on the immune response is not known.

1.4.1.1 Estrogenic Effects on the Innate Immune Response

Estrogens affect the numbers and effector functions of cells involved in innate immunity (Fig. 1.1). For example, 17β-estradiol treatment augments human neutrophil (Nekrasova and Shirshev 2013) and rat mast cell (Vliagoftis et al. 1992) granule release. In mice and rats, the activation of macrophages varies with the estrous cycle, with increasing estradiol enhancing macrophage phagocytosis (Ahmed and Talal 1990; Vernon-Roberts 1969). Estrogen metabolites (e.g.,

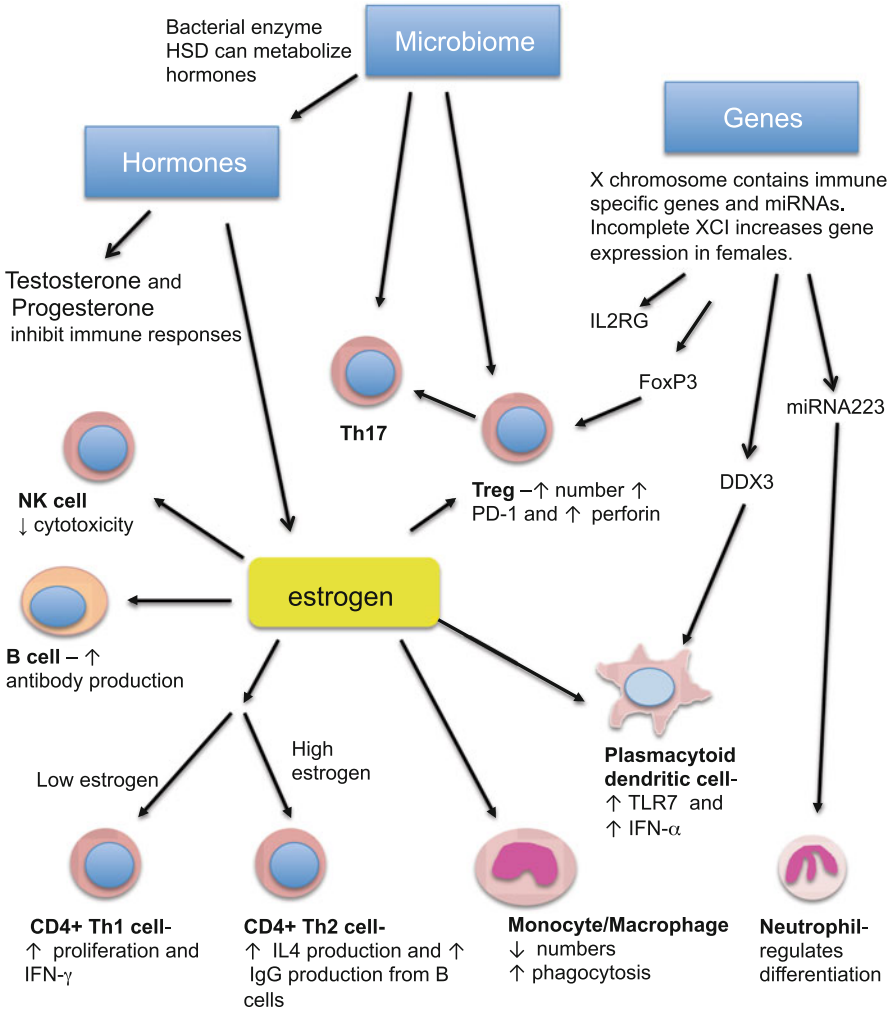

Fig. 1.1 Sex-specific differences influence the immune response. Hormones, genes, and the microbiome all influence the immune response. Estrogens have direct effects on immune cells. Bacterial hydroxysteroid dehydrogenase (HSD), microRNAs (miRNA)

16-hydroxyestrone, 16-hydroxyestradiol, 4-hydroxyestrone, 4-hydroxyestradiol, 2-hydroxyestrone, and 2-hydroxyestradiol) variably affect human monocyte cell proliferation (Capellino et al. 2008) and 17β-estradiol may, under certain conditions, induce cell cycle arrest (Thongngarm et al. 2003). During menopause, when estrogen levels diminish, there is evidence for a significant increase in monocyte numbers (Ben-Hur et al. 1995), whereas individuals on 17β-estradiol therapy exhibit reduced monocyte numbers (Ben-Hur et al. 1995). There are conflicting reports on the effects of 17β-estradiol on murine macrophage activation in response to LPS, with evidence for higher levels of NFκB transcriptional activity and IL-1β, IL-6, and TNF-α production in tissue macrophages (Calippe et al. 2008) as well as evidence for reduced IL-1α, IL-6, and TNF-α production in splenic macrophages (Deshpande et al. 1997). 17β-estradiol suppresses the expression of CD16 (FcγRIIIA, a receptor associated with IgG production and cytokine secretion) on human monocytes and macrophages (Kramer et al. 2004). By contrast, 17-β-estradiol enhances rat macrophage phagocytosis and oxygen radical production (Chao et al. 1994).

There are many reports on the effects of estrogens on NK cells in mouse experimental models of viral infection and tumor progression. In the majority of these studies, a decrease in NK cell activity occurs in mice receiving 17β-estradiol (Nilsson and Carlsten 1994). Yet there are reports that sustained 17β-estradiol treatment in vivo can enhance murine NK cell activity (Screpanti et al. 1987) and that 17β-estradiol enhances human NK cell proliferation in vitro (Sorachi et al. 1993). Interestingly, high serum 17β-estradiol levels in humans correlate with low NK cell cytotoxicity in some diseases (Roszkowski et al. 1993; Provinciali et al. 1995).

Estrogens also influence pDCs. In a humanized mouse model, where the mice express human bone marrow and circulating leukocytes, TLR7 ligation enhanced pDC expression of IFN-α and TNF-α in females compared to males (Seillet et al. 2012). These effects were mediated by estrogens, because deletion of ERα in pDCs blocked these responses (Seillet et al. 2012). Indeed, postmenopausal women treated with 17β-estradiol exhibit markedly enhanced TLR7- and TLR9-dependent production of IFN-α compared with males (Seillet et al. 2012). Both IFN-α and IFN-γ can upregulate the expression of ERs in murine breast cancer cells and thereby initiate a positive regulatory loop (Panchanathan et al. 2010).

1.4.1.2 Estrogenic Effects on the Adaptive Immune Response

Mature lymphocytes express GPER that can trigger an increased calcium flux following antigen presentation, reflective of activation (Benten et al. 1998). Moreover, estrogens influence the maturation of both T and B lymphocytes (Ackerman 2006) (Fig. 1.1).

CD4 and CD8 T-lymphocyte development occurs in the thymus. 17β-estradiol treatment in rats promotes greater percentages of CD4 + CD8+ cells, whereas ovariectomy results in an increased percentage of CD4-CD8+ cells in the thymus

(Shames 2002; Tanriverdi et al. 2003; Leposavic et al. 2001). Estrogens decrease CD4+/CD8+ T-cell development and promote T-cell lymphopoiesis in the liver, which bypasses the negative selection process in the thymus and may promote autoimmunity (Grimaldi et al. 2005; Verthelyi 2001). There is evidence that treatment of human T cells with 17β-estradiol results in a dose-dependent decrease in IL-2 production by T cells (Moulton et al. 2012). While both male and female T cells express ERs, the effect of 17β-estradiol on IL-2 production was more prominent in lymphocytes derived from human females than from males (Moulton et al. 2012). By contrast, 17β-estradiol may also support T-cell survival by increasing Bcl2 expression (Verthelyi 2001).

Estrogens exert immunomodulatory effects on CD4+ T cells (Fig. 1.1). Estrogens regulate Th1 and Th2 responses in a biphasic manner during the menstrual cycle: low doses of estrogens during the luteal phase trigger Th1 cell-mediated immune responses, whereas higher doses during the follicular phase trigger Th2-mediated humoral responses (Pernis 2007). These data suggest that lower levels of estrogens lead to increased expression of the master regulator of Th1 cell differentiation, T-bet (Karpuzoglu et al. 2007). Lower doses of estrogens are associated with enhanced IFN-γ production. IFN-γ transcription may be modified by ERs binding to an estrogen response element (ERE) in the 5' flanking region of this gene (Fox et al. 1991). Additionally, exposing T cells to estrogens can increase responsiveness to IL-12 by increasing STAT4 activation. Notably, under circumstances of high doses of estrogens, such as during pregnancy, IFN-γ production is reduced (Karpuzoglu and Zouali 2011). Higher doses of exogenous 17β-estradiol promote Th2 responses, which increase IL-4, IL-5, and IL-10 levels (Bouman et al. 2005; Ackerman 2006; Zandman-Goddard et al. 2007; Cai et al. 2012). Higher levels of estrogens appear linked to the downregulation of the transcription factor, IRF1, which contributes to Th2 polarization. Specifically, IRF1 regulates IFN-γ production, which in turn suppresses IL-4 transcription (reviewed in (Fish 2008)).

Th17 cells are pro-inflammatory CD4+ lymphocytes that express the transcription factor RORγt and secrete IL-17 (Korn et al. 2007). Recent evidence suggests that estrogens may regulate Th17 lineage commitment. ERα signaling is required for limiting Th1 and Th17 responses in experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis (Lelu et al. 2011; Dunn et al. 2007). One regulatory effect of 17β-estradiol treatment in murine EAE is an increase in the expression of programmed death-1 (PD-1) on regulatory T (Treg) cells and its ligand, PD-L1, on regulatory B cells (Wang et al. 2009; Subramanian et al. 2011). PD-1-PD-L1 interactions occur to limit the proliferation of T cells, thereby suppressing the Th17 pro-inflammatory response. Contradictory mouse studies have suggested that 17β-estradiol treatment of mouse splenocytes increases IL-17 levels (Khan et al. 2010). Estrogenic effects on Th17 cells may also be dose dependent.

Treg cells have important immunoregulatory functions, including control of the size of the peripheral T-cell pool, maintaining self-tolerance by controlling the expansion of autoreactive T cells, and contributing to the tolerance of the semi-allogeneic fetus during pregnancy. Human females have been reported to have lower numbers of Tregs than males (Afshan et al. 2012). In mice, 17β-estradiol can

drive expansion of the Treg in the spleens of mice with EAE (Polanczyk et al. 2004). In humans, the Treg population increases in the peripheral blood during the follicular phase of the menstrual cycle, when estrogen levels are high, and decreases during the luteal phase, when estrogen levels are low (Arruvito et al. 2007). In pregnant mice, Treg cell numbers in the blood, lymph nodes, spleen, thymus, and decidua increase, to maintain tolerance of the fetus (Thuere et al. 2007). In humans, estrogens induce proliferation of peripheral Tregs early in pregnancy (Sasaki et al. 2004), but Treg numbers decline in the second trimester in response to the increasing levels of progesterone (Mjosberg et al. 2009). Estrogens also modify the functional capacity of Tregs. Tregs stimulate inhibitory receptors on other effector T cells and release granules that are cytotoxic. 17-β-estradiol increases the expression of perforin in Tregs, a molecule that punctures the target cell membrane to induce cell death (Valor et al. 2011). 17β-estradiol also increases the suppressive effects of Tregs by inducing the production of the regulatory cytokines IL-10 and TGF-β (Luo et al. 2011) and increasing the surface expression of the inhibitory co-stimulatory molecule, PD-1 (Wang et al. 2009).

Estrogens also affect B cells. B-cell numbers are not affected by fluctuations in estrogens during the menstrual cycle or following hormone replacement therapy (Auerbach et al. 2002). However, the prolonged use of hormone replacement therapy in humans significantly increased B-cell numbers (Porter et al. 2001). Estrogens can reduce the number of bone marrow stromal cells and promote extramedullary B-cell lymphopoiesis (Bouman et al. 2005; Ackerman 2006), potentially bypassing developmental deletion. 17β-estradiol has been shown to increase the percentage of B cells recognizing self-DNA (Grimaldi et al. 2001). 17β-estradiol and prolactin simulate B cells to increase antibody production (Grimaldi et al. 2005; Orbach and Shoenfeld 2007). It has been suggested that estrogens act on B-cell development in the periphery, subsequently increasing the levels of immunoglobulins (Cohen-Solal et al. 2006). 17β-estradiol-mediated Th2 production of IL-4, IL-5, and IL-10 (Bouman et al. 2005; Ackerman 2006; Zandman-Goddard et al. 2007) may be driving B-cell proliferation and maturation to plasma cells (Grimaldi et al. 2005).

1.4.1.3 Progesterone Effects on the Immune System

Progesterone also plays a role in modulating the immune system, yet the evidence for progesterone receptors on immune cells is inconsistent (reviewed in (Dressing et al. 2011)). In contrast to estrogens, progesterone levels peak during the luteal phase of the menstrual cycle and also during pregnancy (Sader et al. 2005; Bouman et al. 2005). Progesterone receptor activation drives Th2 responses (Szekeres-Bartho et al. 2001). Additionally, high progesterone levels during pregnancy reduce NK cell cytotoxicity (Baley and Schacter 1985; Furukawa et al. 1984; Toder et al. 1984a, b). Progesterone can bind to both surface and intracellular receptors (Hughes 2012). Membrane progesterone receptors were identified on murine macrophage cell line- and murine bone marrow-derived macrophages (Dressing

et al. 2011). Intracellular progesterone receptors are found in subsets of NK cells and tissue macrophages (Gilliver 2010). T lymphocytes express cell surface progesterone receptors (Gilliver 2010; Dosiou et al. 2008) and CD4+ T cells express intracellular progesterone receptors (Hughes et al. 2011). Progesterone treatment decreased nitric oxide production and cytokine secretion in murine macrophages (Miller et al. 1996) and inhibited IFN-α production in murine pDCs (Hughes et al. 2008). Progesterone suppresses superoxide release by cells, suppresses perforin expression, and antagonizes chemotaxis induced by estrogens (Munoz-Cruz et al. 2011; Bouman et al. 2005; Laskarin et al. 1999). However, in combination with 17β-estradiol, progesterone can enhance eosinophil degranulation (Hamano et al. 1998). In other studies, progesterone decreases pro-inflammatory cytokine secretion, MHC-II expression, and co-stimulatory markers in female rodent DCs to a greater degree than in male-derived cells (Butts et al. 2008). Progesterone also decreases antibody production by B cells (Lu et al. 2002). Interestingly, progesterone can stimulate T cells derived from human fetal cord blood to differentiate into Tregs but suppresses their differentiation into Th17 cells (Lee et al. 2011).

1.4.1.4 Androgenic Effects on the Immune Cells

Androgens, including dihydrotestosterone and testosterone, exert their action by binding to the intracellular androgen receptor. Once activated, the androgen receptor can induce gene expression directly and modulate signal transduction cascades (Koryakina et al. 2014). Interestingly, among humans, males with lower levels of testosterone may be more prone to autoimmune diseases than those with higher testosterone levels (Tengstrand et al. 2002; Spector et al. 1988; Masi et al. 1999). Testosterone reduces lymphocyte proliferation in response to tuberculin purified protein derivative (Ahmed et al. 1987). Peroxisome proliferator-activated receptor α (PPARα) is a transcription factor that alters the expression of a large number of target genes. PPARα levels are higher in CD4+ T cells from male than female mice and are inducible by testosterone treatment in female mice. Higher PPARα levels correlate with lower T-cell activation and higher Th2 cytokine production in male mice (Dunn et al. 2007). Androgen receptors are expressed in B lymphocytes (Sader et al. 2005) and testosterone therapy decreases antibody levels, which may be linked to the lower prevalence of many autoimmune diseases in men (Bouman et al. 2005; Ackerman 2006). Testosterone therapy in women has been associated with modest clinical benefits in the treatment of autoimmune disease; however, considerable undesirable side effects were reported (Booji et al. 1996). In a recent human study, males with the highest testosterone levels were shown to have the lowest antibody responses to trivalent seasonal influenza vaccine when compared with either females or males with lower circulating testosterone levels (Furman et al. 2013). It was suggested that testosterone-regulated lipid metabolism contributed to this outcome. Testosterone enhances a Th1 response and the activation of CD8+ cells (Bouman et al. 2005; Ackerman 2006; Zandman-Goddard et al. 2007).

Additionally, testosterone increases IL-2 production and clonal expansion of CD8+ cells (Ackerman 2006).

1.4.1.5 Prolactin Effects on the Immune Cells

Prolactin is also associated with regulating immune responses. Prolactin receptors are found on T and B lymphocytes (McMurray 2001) and their activation induces gene transcription, T-cell proliferation, and antibody secretion (Saha et al. 2011; Orbach and Shoenfeld 2007; Bouman et al. 2005; McMurray 2001). Altered prolactin expression has been reported in some SLE patients (Lahita 2000) and in a subset of patients, elevated prolactin levels correlated with high antibody titers and exacerbated disease (Lahita 2000). Prolactin may increase Bcl-2 and CD40 expression in B cells, enhancing their survival (Ackerman 2006; Grimaldi et al. 2005; Saha et al. 2011).

1.4.2 Genomic Effects on Immune Function

While hormones contribute to many of the sex differences in an immune response, the observed immunological differences between prepubertal boys and girls and postmenopausal females compared to elderly males suggest that factors other than hormones influence immune responses in males and females (Lefevre et al. 2012). Certainly, chromosome composition influences immunity: male cells possess one copy each of the X and Y chromosomes, whereas female cells possess two copies of the X chromosome. X-linked genes are associated with disparate immune responses between males and females.

1.4.2.1 Y Chromosome

The X and Y chromosomes were once identical pairs of chromosomes that freely exchanged genetic materials. In mammals, the Y chromosome has evolved to become unique from the X chromosome. It acquired sex-determining regions and underwent many inversions that prevented recombination with the X chromosome and resulted in gene degradation. The Y chromosome is approximately 23 Mb in length and almost exclusively codes for male specific genes (Bachtrog 2013). Since approximately half the population does not have a Y chromosome, it was assumed than no biologically essential genes are present on the Y chromosome. The Y chromosome contains 78 genes. There are several reports of Y-chromosome gene regulation in autoimmunity; however, these were the result of chromosomal translocation of X-chromosome genes (Santiago-Raber et al. 2008; Murphy and Roths 1979). Genetic variation in the Y chromosome affects the susceptibility of male mice to EAE (Teuscher et al. 2006; Spach et al. 2009) as well as contributing to

mortality from coxsackievirus B3 infection (Case et al. 2012). Recently, a mouse with a Y-chromosome-linked defect in NK and B cells has been described, yet the mechanism linked to these defects is not known and sequencing of this Y chromosome has not been performed (Sun et al. 2013). A recent publication by Case et al (Case et al. 2013) has suggested a novel role for the Y chromosome in exerting gene regulatory properties (Case et al. 2013). The copy number of specific male genes inversely correlated with the upregulation of genes in immune cells (Case et al. 2013). These male gene-specific regions contain tandemly repeated DNA elements that may sequester proteins involved in chromatin dynamics. This results in less protein available for chromatin remodeling, reduced euchromatin, and decreased transcriptional activity. Similar observations have been made in a *Drosophila* (Lemos et al. 2013).

1.4.2.2 X Chromosome

In contrast to the Y chromosome, the X chromosome is much larger (150 Mb) and contains 1,100 genes (reviewed in (Fish 2008)). Notably, genes important for reproduction, brain function (Graves 2006), as well as immune regulation (Bianchi et al. 2012) are overrepresented on the X chromosome. X-chromosome inactivation (XCI) occurs in female somatic cells during embryonic development (Lee and Bartolomei 2013)), to compensate for X-gene dosage differences between XX females and XY males. This process is presumably random, is clonally maintained, and results in mosaic expression of either the maternal (Xm) or paternal (Xp) chromosome in different cell populations. In females, skewed XCI may favor the elimination of mutant genes on a single X chromosome. Since male cells express a single X chromosome, no such inactivation can occur; hence, males are more susceptible to gene mutations. Many genes involved in the regulation of the immune system are found on the X chromosome. These include receptors and related proteins and immune response-related genes (reviewed in (Fish 2008)). Consequently, X-linked gene mutations may have a profound impact on immune responses. The most notable example of this is severe combined immunodeficiency (X-SCID), resulting from a mutation in the *IL2RG*, encoding the common gamma chain receptor subunit shared among a number of cytokine receptors: IL-2R, IL-4R, IL-7R, IL-9R, IL-15R, and IL-21R. X-SCID patients lack functional T and B cells and are highly susceptible to infection. X-linked agammaglobulinemia, resulting from a mutation in the Btk gene encoded on the X chromosome, is a consequence of an inability to generate mature B cells and antibodies. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome are caused by mutations in FOXP3, required for Treg lineage commitment (Pinheiro et al. 2011). IPEX individuals suffer from overactive immune responses, resulting in autoimmune conditions. All of these diseases have severe phenotypes in males, with females being relatively unaffected. Specific gene polymorphisms lead to a high-activity X-linked IRAK-variant haplotype, which results in individuals more prone to sepsis (Arcaroli et al. 2006; Toubiana et al. 2010).

Additionally, mosaicism for NOX2 (Chandra et al. 2011) or IRAK protected females from sepsis (Chandra et al. 2013).

The X chromosome contains 7–10 % of all microRNAs (miRNAs) in the genome (Pinheiro et al. 2011; Hewagama et al. 2013), whereas only 2 miRNAs are found on the Y chromosome (Hewagama et al. 2013). miRNAs are small double-stranded noncoding RNAs, and it has been estimated that miRNAs regulate 30–50 % of all protein-coding genes and are involved in the regulation of many cellular processes (Pinheiro et al. 2011). Specific X-chromosome-encoded miRNAs can affect hematopoietic lineage differentiation and cellular activation, thereby modulating the immune response (Lindsay 2008). For example, miR-223 is an X-linked miRNA expressed in the bone marrow and regulates neutrophil differentiation and is significantly reduced in patients with sepsis (Pinheiro et al. 2011). It has been suggested that female mosaicism and different patterns of X-inactivation of miRNAs could lead to sex-specific immune responses (Pinheiro et al. 2011). Several X-linked miRNAs are found in the introns of protein-coding genes, including genes that escape XCI (Pinheiro et al. 2011). It has been suggested that miRNAs may also escape XCI and have aberrant expression patterns (Pinheiro et al. 2011), although this has yet to be demonstrated.

Both XCI and X chromosome upregulation are responsible for regulating gene dosage compensation. Dosage compensation for X-chromosome genes requires upregulation to restore the X to autosome transcription ratio to one in males (Ohno 1967). Many genes can escape XCI and it has been estimated that 10–25 % of the X chromosome escapes inactivation (Lockshin 2010; Carrel and Willard 2005; Prothero et al. 2009; Yang et al. 2010). Notably, in mice XCI occurs in only 3 % of the X chromosome, suggesting that mouse models may not recapitulate gene dosage compensation observed in humans. Additionally, XCI differs in different tissues and among individuals. This may lead to the overproduction of certain gene products in females. The process of XCI is thought to be random; however, as mentioned above, there is evidence for skewed XCI: PBMCs in scleroderma patients exhibit skewed XCI (Oliver and Silman 2009) and XXY males with Klinefelter syndrome have an elevated risk for developing SLE (Dillon et al. 2011).

Twelve genes on the X chromosome have a functional Y counterpart (Wilson and Makova 2009). Most X-linked genes with Y homologues escape XCI (Ross et al. 2005) and there is evidence that these genes have different expression levels as well as different tissue distribution (Wilson and Makova 2009). One notable example is the DDX3 gene, encoding the Dead-box RNA helicase DDX3, which has a role in promoting IFN production and accordingly limits the pathogenesis of HBV and HCV. Females have higher levels of this helicase (Chang et al. 2006), perhaps contributing to their reduced incidence of certain virus infections (Park et al. 2010).

1.5 Microbiome: Sex Hormone Interactions Affect Immune Responses

There is accumulating evidence that the microbiome has a major impact on immunity (Markle and Fish 2013; Cho and Blaser 2012). Polysaccharide A produced by *Bacteroides fragilis* mediates polarization of murine CD4+ T cells into Tregs (Round and Mazmanian 2010) and Th17 cell polarization in the mouse lamina propria requires the presence of segmented filamentous bacteria (Ivanov et al. 2008). Notably, the taxonomy or compositional profile of the microbiome varies by anatomic site. Commensal microorganisms that colonize the gut contribute to the host defense against enteric pathogens (Nicholson et al. 2005). Interestingly, the microbiome in human dizygotic twins of opposite sexes shows a profound sex bias after puberty when compared to age-matched twins of the same sex (Yatsunenko et al. 2012). The implications are that hormones influence the microbiome profile in a sex-specific manner. Bacteria metabolize sex steroids, mediated by hydroxysteroid dehydrogenase (HSD), affecting the balance between active and inactive steroids. The genes for HSD are encoded in the genomes of *Actinobacteria*, *Proteobacteria*, and *Firmicutes*, which colonize the human GI tract (Kisiela et al. 2012). In mice, alteration of the microbiome by antibiotic treatment affects microbial metabolism of sex hormones (Markle et al. 2013) and their subsequent activity. Altering the microbiome in NOD mice affects the incidence of spontaneous type I diabetes development (Markle et al. 2013). Moreover, the female prevalence in this mouse model of autoimmunity is dramatically affected by the microbiome, since in a germ-free environment it is lost. Further, adoptive transfer of commensal bacteria from male mice into young females caused systemic hormonal and metabolic changes and dramatic protection from type 1 diabetes. These changes were not observed in mice lacking the androgen receptor, confirming that protection is sex hormone dependent (Markle et al. 2013).

1.6 Implications for Vaccination and Therapy

Given that females generally mount a stronger humoral immune response to insult compared with males, it is not surprising that human females generate higher antibody titers to vaccines than their male counterparts (Engler et al. 2008; Cook 2008). Human females also consistently report more adverse effects in response to immunization (Klein et al. 2010b). This sex bias is also seen in childhood (Fang et al. 1994). Beyond differences in antibody titers following immunization, there are reports of sex-based differences in innate immune responses following vaccination in humans (Engler et al. 2008). Yellow fever virus vaccine generated IFN production via TLR-activation in human females, but not in males (Klein et al. 2010a). Notably, the HSV-2 vaccine is effective in human females but not in males (Stephenson 2000). The specific mechanisms that contribute to sex

differences in an immune response to pathogen/antigen exposure accompanying immunization—more effective antibody titers and longer-lasting immunity in females, more adverse effects in women—are unclear and will be considered in Chaps. 6 and 10.

1.6.1 Sex Differences in Drug Efficacy and Pharmacokinetics

Given the sex-specific effects on an immune response, it is also imperative that consideration be given to therapeutic interventions—drug treatments—that may also have sex-specific effects. There are notable differences in the levels of many enzymes that affect drug metabolism and also drug bioavailability in males versus females (Franconi et al. 2007) that are beyond the scope of this chapter but are detailed in Chap. 4. Here, we limit the discussion to drugs affecting the immune system. Human females have lower levels of the drug transporter P-glycoprotein, which may contribute to elevated serum levels of certain drugs (Fletcher et al. 2004; Pai et al. 2004). Sex differences in bioavailability have been reported for the immunosuppressive drug, cyclosporine A. Although this was a small cohort and ethnicity also contributed to observed differences, Caucasian women had higher plasma levels of drug than Caucasian men (Min et al. 2000). Similarly, in a rat study following skin grafts, females had higher plasma levels of cyclosporine A compared with males (Enosawa and Hirasawa 1989). The mechanism responsible for this sex disparity is unclear; however, in a rodent study, it was suggested that estrogens did not contribute (Erben et al. 2003). The female sex has been associated with more severe adverse reactions to anti-TNF antibody treatment in human patients with inflammatory bowel disease (Zelinkova et al. 2012; Fidder et al. 2009) and pediatric Crohn's Disease (Crandall and Mackner 2003). Adverse reactions to anti-TNF therapy occur in humans as a result of the development of antibodies against the therapeutic anti-TNF antibodies, which is also associated with loss of efficacy (Radstake et al. 2009; Vultaggio et al. 2010). This antibody response is likely due to the stronger humoral response in females. Nonsteroidal anti-inflammatory drugs have been reported to have reduced efficacy in women compared with men (Kelton et al. 1978). In contrast to reports of the benefits of prophylactic aspirin use in men, the data for women are contradictory, with reports of equal or reduced effectiveness (Eidelman et al. 2003). Nevertheless, sex-based differences in human salicylate metabolism have been identified (Montgomery et al. 1986). Antiretroviral therapy is often more effective in women than men (Gandhi et al. 2004). Females also report more frequent adverse events with protease inhibitors and reverse transcriptase inhibitors (Gandhi et al. 2004). Understanding the differences in the effects of drugs on the immune response in males and females is critical to effective therapy for both males and females and will be considered further in Chap. 4.

1.7 Summary

This chapter highlights the profound effects that sex has on shaping the immune response to pathogen infection, in terms of influencing the incidence of infection mediated by pathogen entry receptors and in terms of influencing disease progression in terms of how the male/female immunophenotype determines the magnitude and quality of the immune response. Genetic, epigenetic, and hormonal influences shape the complement and composition of immune cells and their effector or regulatory functions. Sex-specific differences in the host microbiome also contribute to shaping an immune response. Sex differences in pharmacokinetics and pharmacodynamics of drugs indicate that the same plasma level of a drug does not necessarily result in the same pharmacological outcome. Viewed altogether, accumulating evidence identifies the distinct influences of sex on immune responses that prescribe the pathophysiology of wellness and disease.

References

Ackerman LS (2006) Sex hormones and the genesis of autoimmunity. Arch Dermatol 142 (3):371–376. doi:10.1001/archderm.142.3.371

Afroz R, Hanaki K, Tudin R (2011) Factors affecting waste generation: a study in a waste management program in Dhaka City, Bangladesh. Environ Monit Assess 179(1–4):509–519. doi:10.1007/s10661-010-1753-4

Afshan G, Afzal N, Qureshi S (2012) CD4 + CD25(hi) regulatory T cells in healthy males and females mediate gender difference in the prevalence of autoimmune diseases. Clin Lab 58 (5–6):567–571

Ahmed SA, Talal N (1990) Sex hormones and the immune system–Part 2. Animal data. Baillieres Clin Rheumatol 4(1):13–31. doi:10.1016/S0950-3579(05)80241-9

Ahmed SA, Talal N, Christadoss P (1987) Genetic regulation of testosterone-induced immune suppression. Cell Immunol 104(1):91–98. doi:10.1016/0008-8749(87)90009-8

Amadori A, Zamarchi R, De Silvestro G, Forza G, Cavatton G, Danieli GA, Clementi M, Chieco-Bianchi L (1995) Genetic control of the CD4/CD8 T-cell ratio in humans. Nat Med 1 (12):1279–1283. doi:10.1038/nm1295-1279

Aomatsu M, Kato T, Kasahara E, Kitagawa S (2013) Gender difference in tumor necrosis factor-alpha production in human neutrophils stimulated by lipopolysaccharide and interferon-gamma. Biochem Biophys Res Commun 441(1):220–225. doi:10.1016/j.bbrc.2013.10.042

Arcaroli J, Silva E, Maloney JP, He Q, Svetkauskaite D, Murphy JR, Abraham E (2006) Variant IRAK-1 haplotype is associated with increased nuclear factor-kappaB activation and worse outcomes in sepsis. Am J Respir Crit Care Med 173(12):1335–1341. doi:10.1164/rccm.200603-341OC

Armien B, Pascale JM, Bayard V, Munoz C, Mosca I, Guerrero G, Armien A, Quiroz E, Castillo Z, Zaldivar Y, Gracia F, Hjelle B, Koster F (2004) High seroprevalence of hantavirus infection on the Azuero peninsula of Panama. Am J Trop Med Hyg 70(6):682–687

Arruvito L, Sanz M, Banham AH, Fainboim L (2007) Expansion of CD4 + CD25 + and FOXP3+ regulatory T cells during the follicular phase of the menstrual cycle: implications for human reproduction. J Immunol 178(4):2572–2578. doi:10.4049/jimmunol.178.4.2572

Auerbach L, Hafner T, Huber JC, Panzer S (2002) Influence of low-dose oral contraception on peripheral blood lymphocyte subsets at particular phases of the hormonal cycle. Fertil Steril 78 (1):83–89. doi:10.1016/S0015-0282(02)03173-4

Bachtrog D (2013) Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. Nat Rev Genet 14(2):113–124. doi:10.1038/nrg3366

Baley JE, Schacter BZ (1985) Mechanisms of diminished natural killer cell activity in pregnant women and neonates. J Immunol 134(5):3042–3048

Ben-Hur H, Mor G, Insler V, Blickstein I, Amir-Zaltsman Y, Sharp A, Globerson A, Kohen F (1995) Menopause is associated with a significant increase in blood monocyte number and a relative decrease in the expression of estrogen receptors in human peripheral monocytes. Am J Reprod Immunol 34(6):363–369. doi:10.1111/j.1600-0897.1995.tb00965.x

Benten WP, Lieberherr M, Giese G, Wunderlich F (1998) Estradiol binding to cell surface raises cytosolic free calcium in T cells. FEBS Lett 422(3):349–353, S0014-5793(98)00039-8

Berghofer B, Frommer T, Haley G, Fink L, Bein G, Hackstein H (2006) TLR7 ligands induce higher IFN-alpha production in females. J Immunol 177(4):2088–2096

Bianchi I, Lleo A, Gershwin ME, Invernizzi P (2012) The X chromosome and immune associated genes. J Autoimmun 38(2–3):J187–J192. doi:10.1016/j.jaut.2011.11.012

Blesson CS, Sahlin L (2012) Expression pattern and signalling pathways in neutrophil like HL-60 cells after treatment with estrogen receptor selective ligands. Mol Cell Endocrinol 361 (1–2):179–190. doi:10.1016/j.mce.2012.04.006

Boissier J, Chlichlia K, Digon Y, Ruppel A, Mone H (2003) Preliminary study on sex-related inflammatory reactions in mice infected with Schistosoma mansoni. Parasitol Res 91 (2):144–150. doi:10.1007/s00436-003-0943-1

Booji A, Biewenga-Booji CM, Huber-Bruning O, Cornelis C, Jacobs JW, Bijlsma JW (1996) Androgens as adjuvant treatment in postmenopausal female patients with rheumatoid arthritis. Ann Rheum Dis 55(11):811–815. doi:10.1136/ard.55.11.811

Bouman A, Schipper M, Heineman MJ, Faas MM (2004) Gender difference in the non-specific and specific immune response in humans. Am J Reprod Immunol 52(1):19–26. doi:10.1111/j.1600-0897.2004.00177.x

Bouman A, Heineman MJ, Faas MM (2005) Sex hormones and the immune response in humans. Hum Reprod Update 11(4):411–423. doi:10.1093/humupd/dmi008

Butterworth M, McClellan B, Allansmith M (1967) Influence of sex in immunoglobulin levels. Nature 214(5094):1224–1225. doi:10.1038/2141224a0

Butts CL, Bowers E, Horn JC, Shukair SA, Belyavskaya E, Tonelli L, Sternberg EM (2008) Inhibitory effects of progesterone differ in dendritic cells from female and male rodents. Gend Med 5(4):434–447. doi:10.1016/j.genm.2008.11.001

Cai Y, Zhou J, Webb DC (2012) Estrogen stimulates Th2 cytokine production and regulates the compartmentalisation of eosinophils during allergen challenge in a mouse model of asthma. Int Arch Allergy Immunol 158(3):252–260. doi:10.1159/000331437

Calippe B, Douin-Echinard V, Laffargue M, Laurell H, Rana-Poussine V, Pipy B, Guery JC, Bayard F, Arnal JF, Gourdy P (2008) Chronic estradiol administration in vivo promotes the proinflammatory response of macrophages to TLR4 activation: involvement of the phosphatidylinositol 3-kinase pathway. J Immunol 180(12):7980–7988. doi:10.4049/jimmunol.180.12.7980

Capellino S, Montagna P, Villaggio B, Soldano S, Straub RH, Cutolo M (2008) Hydroxylated estrogen metabolites influence the proliferation of cultured human monocytes: possible role in synovial tissue hyperplasia. Clin Exp Rheumatol 26(5):903–909

Carrel L, Willard HF (2005) X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 434(7031):400–404. doi:10.1038/nature03479

Case LK, Toussaint L, Moussawi M, Roberts B, Saligrama N, Brossay L, Huber SA, Teuscher C (2012) Chromosome Y regulates survival following murine coxsackievirus b3 infection. G3 (Bethesda) 2(1):115–121. doi:10.1534/g3.111.001610

Case LK, Wall EH, Dragon JA, Saligrama N, Krementsov DN, Moussawi M, Zachary JF, Huber SA, Blankenhorn EP, Teuscher C (2013) The Y chromosome as a regulatory element shaping immune cell transcriptomes and susceptibility to autoimmune disease. Genome Res 23 (9):1474–1485. doi:10.1101/gr.156703.113

Cernetich A, Garver LS, Jedlicka AE, Klein PW, Kumar N, Scott AL, Klein SL (2006) Involvement of gonadal steroids and gamma interferon in sex differences in response to blood-stage malaria infection. Infect Immun 74(6):3190–3203. doi:10.1128/IAI. 00008-06

Chandra R, Federici S, Nemeth ZH, Horvath B, Pacher P, Hasko G, Deitch EA, Spolarics Z (2011) Female X-chromosome mosaicism for NOX2 deficiency presents unique inflammatory phenotype and improves outcome in polymicrobial sepsis. J Immunol 186(11):6465–6473. doi:10.4049/jimmunol.1100205

Chandra R, Federici S, Nemeth ZH, Csoka B, Thomas JA, Donnelly R, Spolarics Z (2013) Cellular mosaicism for X-linked polymorphisms and IRAK1 expression presents a distinct phenotype and improves survival following sepsis. J Leukoc Biol 95(3):497–507. doi:10.1189/jlb.0713397

Chang PC, Chi CW, Chau GY, Li FY, Tsai YH, Wu JC, Wu Lee YH (2006) DDX3, a DEAD box RNA helicase, is deregulated in hepatitis virus-associated hepatocellular carcinoma and is involved in cell growth control. Oncogene 25(14):1991–2003. doi:10.1038/sj.onc.1209239

Chao TC, Van Alten PJ, Walter RJ (1994) Steroid sex hormones and macrophage function: modulation of reactive oxygen intermediates and nitrite release. Am J Reprod Immunol 32 (1):43–52. doi:10.1111/j.1600-0897.1994.tb00877.x

Cho I, Blaser MJ (2012) The human microbiome: at the interface of health and disease. Nat Rev Genet 13(4):260–270. doi:10.1038/nrg3182

Cohen-Solal JF, Jeganathan V, Grimaldi CM, Peeva E, Diamond B (2006) Sex hormones and SLE: influencing the fate of autoreactive B cells. Curr Top Microbiol Immunol 305:67–88. doi:10.1007/3-540-29714-6_4

Colasanti T, Maselli A, Conti F, Sanchez M, Alessandri C, Barbati C, Vacirca D, Tinari A, Chiarotti F, Giovannetti A, Franconi F, Valesini G, Malorni W, Pierdominici M, Ortona E (2012) Autoantibodies to estrogen receptor alpha interfere with T lymphocyte homeostasis and are associated with disease activity in systemic lupus erythematosus. Arthritis Rheum 64 (3):778–787. doi:10.1002/art.33400

Cook IF (2008) Sexual dimorphism of humoral immunity with human vaccines. Vaccine 26 (29–30):3551–3555. doi:10.1016/j.vaccine.2008.04.054

Crandall WV, Mackner LM (2003) Infusion reactions to infliximab in children and adolescents: frequency, outcome and a predictive model. Aliment Pharmacol Ther 17(1):75–84. doi:10.1046/j.1365-2036.2003.01411.x

Cunningham M, Gilkeson G (2011) Estrogen receptors in immunity and autoimmunity. Clin Rev Allergy Immunol 40(1):66–73. doi:10.1007/s12016-010-8203-5

Dao H Jr, Kazin RA (2007) Gender differences in skin: a review of the literature. Gend Med 4 (4):308–328. doi:10.1016/S1550-8579(07)80061-1

Das BR, Bhanushali AA, Khadapkar R, Jeswani KD, Bhavsar M, Dasgupta A (2008) Reference ranges for lymphocyte subsets in adults from western India: influence of sex, age and method of enumeration. Indian J Med Sci 62(10):397–406. doi:10.4103/0019-5359.42725

Deshpande R, Khalili H, Pergolizzi RG, Michael SD, Chang MD (1997) Estradiol down-regulates LPS-induced cytokine production and NFkB activation in murine macrophages. Am J Reprod Immunol 38(1):46–54. doi:10.1111/j.1600-0897.1997.tb00275.x

Dillon S, Aggarwal R, Harding JW, Li LJ, Weissman MH, Li S, Cavett JW, Sevier ST, Ojwang JW, D'Souza A, Harley JB, Scofield RH (2011) Klinefelter's syndrome (47, XXY) among men with systemic lupus erythematosus. Acta Paediatr 100(6):819–823. doi:10.1111/j.1651-2227.2011.02185.x

Doeing DC, Borowicz JL, Crockett ET (2003) Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. BMC Clin Pathol 3(1):3. doi:10.1186/1472-6890-3-3

Dosiou C, Hamilton AE, Pang Y, Overgaard MT, Tulac S, Dong J, Thomas P, Giudice LC (2008) Expression of membrane progesterone receptors on human T lymphocytes and Jurkat cells and activation of G-proteins by progesterone. J Endocrinol 196(1):67–77. doi:10.1677/JOE-07-0317

Dressing GE, Goldberg JE, Charles NJ, Schwertfeger KL, Lange CA (2011) Membrane progesterone receptor expression in mammalian tissues: a review of regulation and physiological implications. Steroids 76(1–2):11–17. doi:10.1016/j.steroids.2010.09.006

Dunn SE, Ousman SS, Sobel RA, Zuniga L, Baranzini SE, Youssef S, Crowell A, Loh J, Oksenberg J, Steinman L (2007) Peroxisome proliferator-activated receptor (PPAR)alpha expression in T cells mediates gender differences in development of T cell-mediated autoimmunity. J Exp Med 204(2):321–330. doi:10.1084/jem.20061839

Eidelman RS, Hebert PR, Weisman SM, Hennekens CH (2003) An update on aspirin in the primary prevention of cardiovascular disease. Arch Intern Med 163(17):2006–2010. doi:10.1001/archinte.163.17.2006

Engler RJ, Nelson MR, Klote MM, VanRaden MJ, Huang CY, Cox NJ, Klimov A, Keitel WA, Nichol KL, Carr WW, Treanor JJ (2008) Half- vs full-dose trivalent inactivated influenza vaccine (2004–2005): age, dose, and sex effects on immune responses. Arch Intern Med 168 (22):2405–2414. doi:10.1001/archinternmed.2008.513

Enosawa S, Hirasawa K (1989) Sex-associated differences in the survival of skin grafts in rats. Enhancement of cyclosporine immunosuppression in male compared with female recipients. Transplantation 47(6):933–937. doi:10.1097/00007890-198906000-00002

Erben RG, Brunner KS, Breig B, Eberle J, Goldberg M, Hofbauer LC (2003) Skeletal effects of cyclosporin A are gender related in rats. Endocrinology 144(1):40–49. doi:10.1210/en.2002-220513

Fang JW, Lai CL, Chung HT, Wu PC, Lau JY (1994) Female children respond to recombinant hepatitis B vaccine with a higher titre than male. J Trop Pediatr 40(2):104–107. doi:10.1093/tropej/40.2.104

Farzadegan H, Hoover DR, Astemborski J, Lyles CM, Margolick JB, Markham RB, Quinn TC, Vlahov D (1998) Sex differences in HIV-1 viral load and progression to AIDS. Lancet 352 (9139):1510–1514. doi:10.1016/S0140-6736(98)02372-1

Fidder H, Schnitzler F, Ferrante M, Noman M, Katsanos K, Segaert S, Henckaerts L, Van Assche G, Vermeire S, Rutgeerts P (2009) Long-term safety of infliximab for the treatment of inflammatory bowel disease: a single-centre cohort study. Gut 58(4):501–508. doi:10.1136/gut.2008.163642

Fish EN (2008) The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol 8(9):737–744. doi:10.1038/nri2394

Fletcher CV, Jiang H, Brundage RC, Acosta EP, Haubrich R, Katzenstein D, Gulick RM (2004) Sex-based differences in saquinavir pharmacology and virologic response in AIDS Clinical Trials Group Study 359. J Infect Dis 189(7):1176–1184. doi:10.1086/382754

Fox HS, Bond BL, Parslow TG (1991) Estrogen regulates the IFN-gamma promoter. J Immunol 146(12):4362–4367

Franconi F, Brunelleschi S, Steardo L, Cuomo V (2007) Gender differences in drug responses. Pharmacol Res 55(2):81–95. doi:10.1016/j.phrs.2006.11.001

Furman D, Hejblum BP, Simon N, Jojic V, Dekker CL, Thiebaut R, Tibshirani RJ, Davis MM (2013) Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. Proc Natl Acad Sci U S A 111(2):869–874. doi:10.1073/pnas.1321060111

Furukawa K, Itoh K, Okamura K, Kumagai K, Suzuki M (1984) Changes in NK cell activity during the estrous cycle and pregnancy in mice. J Reprod Immunol 6(6):353–363. doi:10.1016/0165-0378(84)90045-7

Gandhi M, Aweeka F, Greenblatt RM, Blaschke TF (2004) Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol 44:499–523. doi:10.1146/annurev.pharmtox.44.101802.121453

Gavrilovskaya IN, Shepley M, Shaw R, Ginsberg MH, Mackow ER (1998) beta3 Integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc Natl Acad Sci U S A 95 (12):7074–7079

Gilliet M, Cao W, Liu YJ (2008) Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Nat Rev Immunol 8(8):594–606. doi:10.1038/nri2358

Gilliver SC (2010) Sex steroids as inflammatory regulators. J Steroid Biochem Mol Biol 120 (2–3):105–115. doi:10.1016/j.jsbmb.2009.12.015

Gleicher N, Barad DH (2007) Gender as risk factor for autoimmune diseases. J Autoimmun 28 (1):1–6. doi:10.1016/j.jaut.2006.12.004

Graves JA (2006) Sex chromosome specialization and degeneration in mammals. Cell 124 (5):901–914. doi:10.1016/j.cell.2006.02.024

Grimaldi CM, Michael DJ, Diamond B (2001) Cutting edge: expansion and activation of a population of autoreactive marginal zone B cells in a model of estrogen-induced lupus. J Immunol 167(4):1886–1890. doi:10.4049/jimmunol.167.4.1886

Grimaldi CM, Hill L, Xu X, Peeva E, Diamond B (2005) Hormonal modulation of B cell development and repertoire selection. Mol Immunol 42(7):811–820. doi:10.1016/j.molimm.2004.05.014

Hamano N, Terada N, Maesako K, Numata T, Konno A (1998) Effect of sex hormones on eosinophilic inflammation in nasal mucosa. Allergy Asthma Proc 19(5):263–269. doi:10.2500/108854198778557773

Hannah MF, Bajic VB, Klein SL (2008) Sex differences in the recognition of and innate antiviral responses to Seoul virus in Norway rats. Brain Behav Immun 22(4):503–516. doi:10.1016/j.bbi.2007.10.005

Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Strom A, Treuter E, Warner M, Gustafsson JA (2007) Estrogen receptors: how do they signal and what are their targets. Physiol Rev 87(3):905–931. doi:10.1152/physrev.00026.2006

Hewagama A, Patel D, Yarlagadda S, Strickland FM, Richardson BC (2009) Stronger inflammatory/cytotoxic T-cell response in women identified by microarray analysis. Genes Immun 10 (5):509–516. doi:10.1038/gene.2009.12

Hewagama A, Gorelik G, Patel D, Liyanarachchi P, McCune WJ, Somers E, Gonzalez-Rivera T, Strickland F, Richardson B (2013) Overexpression of X-linked genes in T cells from women with lupus. J Autoimmun 41:60–71. doi:10.1016/j.jaut.2012.12.006

Hughes GC (2012) Progesterone and autoimmune disease. Autoimmun Rev 11(6–7):A502–A514. doi:10.1016/j.autrev.2011.12.003

Hughes GC, Thomas S, Li C, Kaja MK, Clark EA (2008) Cutting edge: progesterone regulates IFN-alpha production by plasmacytoid dendritic cells. J Immunol 180(4):2029–2033. doi:10.4049/ jimmunol.180.4.2029

Hughes GC, Clark EA, Wong AH (2011) The intracellular progesterone receptor regulates CD4+ T cells and T cell-dependent antibody responses. J Leukoc Biol 93(3):369–375. doi:10.1189/jlb.1012491

Ivanov II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR (2008) Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe 4(4):337–349. doi:10.1016/j.chom.2008.09.009

Jaques R, Ruegg M (1970) Age and sex differences in mast cell count and histamine content. Agents Actions 1(3):144–147. doi:10.1007/BF01982401

Jean CM, Honarmand S, Louie JK, Glaser CA (2007) Risk factors for West Nile virus neuroinvasive disease, California, 2005. Emerg Infect Dis 13(12):1918–1920. doi:10.3201/eid1312.061265

Karpuzoglu E, Zouali M (2011) The multi-faceted influences of estrogen on lymphocytes: toward novel immuno-interventions strategies for autoimmunity management. Clin Rev Allergy Immunol 40(1):16–26. doi:10.1007/s12016-009-8188-0

Karpuzoglu E, Phillips RA, Gogal RM Jr, Ansar Ahmed S (2007) IFN-gamma-inducing transcription factor, T-bet is upregulated by estrogen in murine splenocytes: role of IL-27 but not IL-12. Mol Immunol 44(7):1808–1814. doi:10.1016/j.molimm.2006.08.005

Kee SJ, Park YW, Cho YN, Jin HM, Kim MJ, Lee SJ, Kim TJ, Lee SS, Kwon YS, Jang HC, Kim N, Shin MG, Shin JH, Suh SP, Ryang DW (2012) Age- and gender-related differences in circulating natural killer T cells and their subset levels in healthy Korean adults. Hum Immunol 73(10):1011–1016. doi:10.1016/j.humimm.2012.07.335

Kelton JG, Hirsh J, Carter CJ, Buchanan MR (1978) Sex differences in the antithrombotic effects of aspirin. Blood 52(5):1073–1076

Khan D, Dai R, Karpuzoglu E, Ahmed SA (2010) Estrogen increases, whereas IL-27 and IFN-gamma decrease, splenocyte IL-17 production in WT mice. Eur J Immunol 40 (9):2549–2556. doi:10.1002/eji.201040303

Kisiela M, Skarka A, Ebert B, Maser E (2012) Hydroxysteroid dehydrogenases (HSDs) in bacteria: a bioinformatic perspective. J Steroid Biochem Mol Biol 129(1–2):31–46. doi:10.1016/j.jsbmb.2011.08.002

Klein SL (2000) The effects of hormones on sex differences in infection: from genes to behavior. Neurosci Biobehav Rev 24(6):627–638. doi:10.1016/S0149-7634(00)00027-0

Klein SL (2012a) Immune cells have sex and so should journal articles. Endocrinology 153 (6):2544–2550. doi:10.1210/en.2011-2120

Klein SL (2012b) Sex influences immune responses to viruses, and efficacy of prophylaxis and treatments for viral diseases. Bioessays 34(12):1050–1059. doi:10.1002/bies.201200099

Klein SL, Jedlicka A, Pekosz A (2010a) The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis 10(5):338–349. doi:10.1016/S1473-3099(10)70049-9

Klein SL, Passaretti C, Anker M, Olukoya P, Pekosz A (2010b) The impact of sex, gender and pregnancy on 2009 H1N1 disease. Biol Sex Differ 1(1):5. doi:10.1186/2042-6410-1-5

Korn T, Oukka M, Kuchroo V, Bettelli E (2007) Th17 cells: effector T cells with inflammatory properties. Semin Immunol 19(6):362–371. doi:10.1016/j.smim.2007.10.007

Koryakina Y, Ta HQ, Gioeli D (2014) Androgen receptor phosphorylation: biological context and functional consequences. Endocr Relat Cancer 21:T131–T145. doi:10.1530/ERC-13-0472

Kramer PR, Kramer SF, Guan G (2004) 17 beta-estradiol regulates cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages. Arthritis Rheum 50(6):1967–1975. doi:10.1002/art.20309

Lahita RG (2000) Sex hormones and systemic lupus erythematosus. Rheum Dis Clin North Am 26 (4):951–968. doi:10.1016/S0889-857X(05)70178-2

Laskarin G, Strbo N, Sotosek V, Rukavina D, Faust Z, Szekeres-Bartho J, Podack ER (1999) Progesterone directly and indirectly affects perforin expression in cytolytic cells. Am J Reprod Immunol 42(5):312–320. doi:10.1111/j.1600-0897.1999.tb00107.x

Lee JT, Bartolomei MS (2013) X-inactivation, imprinting, and long noncoding RNAs in health and disease. Cell 152(6):1308–1323. doi:10.1016/j.cell.2013.02.016

Lee JH, Ulrich B, Cho J, Park J, Kim CH (2011) Progesterone promotes differentiation of human cord blood fetal T cells into T regulatory cells but suppresses their differentiation into Th17 cells. J Immunol 187(4):1778–1787. doi:10.4049/jimmunol.1003919

Lefevre N, Corazza F, Duchateau J, Desir J, Casimir G (2012) Sex differences in inflammatory cytokines and CD99 expression following in vitro lipopolysaccharide stimulation. Shock 38 (1):37–42. doi:10.1097/SHK.0b013e3182571e46

Lelu K, Laffont S, Delpy L, Paulet PE, Perinat T, Tschanz SA, Pelletier L, Engelhardt B, Guery JC (2011) Estrogen receptor alpha signaling in T lymphocytes is required for estradiol-mediated inhibition of Th1 and Th17 cell differentiation and protection against experimental autoimmune encephalomyelitis. J Immunol 187(5):2386–2393. doi:10.4049/jimmunol.1101578

Lemos B, Branco AT, Hartl DL (2013) Epigenetic effects of polymorphic Y chromosomes modulate chromatin components, immune response, and sexual conflict. Proc Natl Acad Sci U S A 107(36):15826–15831. doi:10.1073/pnas.1010383107

Leone M, Honstettre A, Lepidi H, Capo C, Bayard F, Raoult D, Mege JL (2004) Effect of sex on Coxiella burnetii infection: protective role of 17beta-estradiol. J Infect Dis 189(2):339–345. doi:10.1086/380798

Leposavic G, Obradovic S, Kosec D, Pejcic-Karapetrovic B, Vidic-Dankovic B (2001) In vivo modulation of the distribution of thymocyte subsets by female sex steroid hormones. Int Immunopharmacol 1(1):1–12. doi:10.1016/S1567-5769(00)00006-0

Lindsay MA (2008) microRNAs and the immune response. Trends Immunol 29(7):343–351. doi:10.1016/j.it.2008.04.004

Lockshin MD (2010) Nonhormonal explanations for sex discrepancy in human illness. Ann N Y Acad Sci 1193:22–24. doi:10.1111/j.1749-6632.2009.05293.x

Lu FX, Abel K, Ma Z, Rourke T, Lu D, Torten J, McChesney M, Miller CJ (2002) The strength of B cell immunity in female rhesus macaques is controlled by CD8+ T cells under the influence of ovarian steroid hormones. Clin Exp Immunol 128(1):10–20. doi:10.1046/j.1365-2249.2002.01780.x

Luo CY, Wang L, Sun C, Li DJ (2011) Estrogen enhances the functions of CD4(+)CD25(+)Foxp3(+) regulatory T cells that suppress osteoclast differentiation and bone resorption in vitro. Cell Mol Immunol 8(1):50–58. doi:10.1038/cmi.2010.54

Markle JG, Fish EN (2013) SeXX matters in immunity. Trends Immunol 35(3):97–104. doi:10.1016/j.it.2013.10.006

Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, von Bergen M, McCoy KD, Macpherson AJ, Danska JS (2013) Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science 339(6123):1084–1088. doi:10.1126/science.1233521

Masi AT, Bijlsma JW, Chikanza IC, Pitzalis C, Cutolo M (1999) Neuroendocrine, immunologic, and microvascular systems interactions in rheumatoid arthritis: physiopathogenetic and therapeutic perspectives. Semin Arthritis Rheum 29(2):65–81. doi:10.1016/S0049-0172(99)80039-0

McMurray RW (2001) Estrogen, prolactin, and autoimmunity: actions and interactions. Int Immunopharmacol 1(6):995–1008. doi:10.1016/S1567-5769(01)00045-5

Meier A, Chang JJ, Chan ES, Pollard RB, Sidhu HK, Kulkarni S, Wen TF, Lindsay RJ, Orellana L, Mildvan D, Bazner S, Streeck H, Alter G, Lifson JD, Carrington M, Bosch RJ, Robbins GK, Altfeld M (2009) Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. Nat Med 15(8):955–959. doi:10.1038/nm.2004

Melgert BN, Oriss TB, Qi Z, Dixon-McCarthy B, Geerlings M, Hylkema MN, Ray A (2010) Macrophages: regulators of sex differences in asthma? Am J Respir Cell Mol Biol 42(5):595–603. doi:10.1165/rcmb.2009-0016OC

Miller L, Alley EW, Murphy WJ, Russell SW, Hunt JS (1996) Progesterone inhibits inducible nitric oxide synthase gene expression and nitric oxide production in murine macrophages. J Leukoc Biol 59(3):442–450

Min DI, Lee M, Ku YM, Flanigan M (2000) Gender-dependent racial difference in disposition of cyclosporine among healthy African American and white volunteers. Clin Pharmacol Ther 68(5):478–486. doi:10.1067/mcp.2000.111255

Mjosberg J, Svensson J, Johansson E, Hellstrom L, Casas R, Jenmalm MC, Boij R, Matthiesen L, Jonsson JI, Berg G, Ernerudh J (2009) Systemic reduction of functionally suppressive CD4dimCD25highFoxp3+ Tregs in human second trimester pregnancy is induced by progesterone and 17beta-estradiol. J Immunol 183(1):759–769. doi:10.4049/jimmunol.0803654

Mo R, Chen J, Grolleau-Julius A, Murphy HS, Richardson BC, Yung RL (2005) Estrogen regulates CCR gene expression and function in T lymphocytes. J Immunol 174(10):6023–6029. doi:10.4049/jimmunol.174.10.6023

Montgomery PR, Berger LG, Mitenko PA, Sitar DS (1986) Salicylate metabolism: effects of age and sex in adults. Clin Pharmacol Ther 39(5):571–576. doi:10.1038/clpt.1986.98

Montoya CJ, Pollard D, Martinson J, Kumari K, Wasserfall C, Mulder CB, Rugeles MT, Atkinson MA, Landay AL, Wilson SB (2007) Characterization of human invariant natural killer T

subsets in health and disease using a novel invariant natural killer T cell-clonotypic monoclonal antibody, 6B11. Immunology 122(1):1–14. doi:10.1111/j.1365-2567.2007.02647.x

Moulton VR, Holcomb DR, Zajdel MC, Tsokos GC (2012) Estrogen upregulates cyclic AMP response element modulator alpha expression and downregulates interleukin-2 production by human T lymphocytes. Mol Med 18:370–378. doi:10.2119/molmed.2011.00506

Munoz-Cruz S, Togno-Pierce C, Morales-Montor J (2011) Non-reproductive effects of sex steroids: their immunoregulatory role. Curr Top Med Chem 11(13):1714–1727. doi:10.2174/156802611796117630

Murphy ED, Roths JB (1979) A Y chromosome associated factor in strain BXSB producing accelerated autoimmunity and lymphoproliferation. Arthritis Rheum 22(11):1188–1194. doi:10.1002/art.1780221105

Napravnik S, Poole C, Thomas JC, Eron JJ Jr (2002) Gender difference in HIV RNA levels: a meta-analysis of published studies. J Acquir Immune Defic Syndr 31(1):11–19. doi:10.1097/00126334-200209010-00002

Nekrasova IV, Shirshev SV (2013) Female sex steroid hormones in regulation of neutrophil enzymatic activity. Dokl Biochem Biophys 453(1):312–315. doi:10.1134/S1607672913060100

Neyrolles O, Quintana-Murci L (2009) Sexual inequality in tuberculosis. PLoS Med 6(12): e1000199. doi:10.1371/journal.pmed.1000199

Nicholson JK, Holmes E, Wilson ID (2005) Gut microorganisms, mammalian metabolism and personalized health care. Nat Rev Microbiol 3(5):431–438. doi:10.1038/nrmicro1152

Nilsson N, Carlsten H (1994) Estrogen induces suppression of natural killer cell cytotoxicity and augmentation of polyclonal B cell activation. Cell Immunol 158(1):131–139. doi:10.1006/cimm.1994.1262

Ohno S (1967) Sex chromosomes and sex-linked genes. Springer, Berlin

Oliver JE, Silman AJ (2009) Why are women predisposed to autoimmune rheumatic diseases? Arthritis Res Ther 11(5):252. doi:10.1186/ar2825

Orbach H, Shoenfeld Y (2007) Hyperprolactinemia and autoimmune diseases. Autoimmun Rev 6 (8):537–542. doi:10.1016/j.autrev.2006.10.005

Owman C, Blay P, Nilsson C, Lolait SJ (1996) Cloning of human cDNA encoding a novel heptahelix receptor expressed in Burkitt's lymphoma and widely distributed in brain and peripheral tissues. Biochem Biophys Res Commun 228(2):285–292. doi:10.1006/bbrc.1996.1654

Pai MP, Schriever CA, Diaz-Linares M, Novak RM, Rodvold KA (2004) Sex-related differences in the pharmacokinetics of once-daily saquinavir soft-gelatin capsules boosted with low-dose ritonavir in patients infected with human immunodeficiency virus type 1. Pharmacotherapy 24 (5):592–599. doi:10.1592/phco.24.6.592.34744

Panchanathan R, Shen H, Zhang X, Ho SM, Choubey D (2010) Mutually positive regulatory feedback loop between interferons and estrogen receptor-alpha in mice: implications for sex bias in autoimmunity. PLoS One 5(5):e10868. doi:10.1371/journal.pone.0010868

Park C, Carrel L, Makova KD (2010) Strong purifying selection at genes escaping X chromosome inactivation. Mol Biol Evol 27(11):2446–2450. doi:10.1093/molbev/msq143

Pathak S, Rege M, Gogtay NJ, Aigal U, Sharma SK, Valecha N, Bhanot G, Kshirsagar NA, Sharma S (2012) Age-dependent sex bias in clinical malarial disease in hypoendemic regions. PLoS One 7(4):e35592. doi:10.1371/journal.pone.0035592

Pennell LM, Galligan CL, Fish EN (2012) Sex affects immunity. J Autoimmun 38(2–3):J282–J291. doi:10.1016/j.jaut.2011.11.013

Pernis AB (2007) Estrogen and CD4+ T cells. Curr Opin Rheumatol 19(5):414–420. doi:10.1097/BOR.0b013e328277ef2a

Peters LL Barker JE () Hematology and clotting time survey in 43 inbred strains of mice. MPD: Peters1. Mouse Phenome Database web site, The Jackson Laboratory, Bar Harbor, Maine USA. http://phenome.jax.org, Feb, 2014.

Pinheiro I, Dejager L, Libert C (2011) X-chromosome-located microRNAs in immunity: might they explain male/female differences?: the X chromosome-genomic context may affect X-located miRNAs and downstream signaling, thereby contributing to the enhanced immune response of females. Bioessays 33(11):791–802. doi:10.1002/bies.201100047

Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S (2006) Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science 312(5780):1669–1672. doi:10.1126/science.1124978

Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, Offner H (2004) Cutting edge: estrogen drives expansion of the CD4 + CD25+ regulatory T cell compartment. J Immunol 173(4):2227–2230. doi:10.4049/jimmunol.173.4.2227

Porter VR, Greendale GA, Schocken M, Zhu X, Effros RB (2001) Immune effects of hormone replacement therapy in post-menopausal women. Exp Gerontol 36(2):311–326. doi:10.1016/S0531-5565(00)00195-9

Prothero KE, Stahl JM, Carrel L (2009) Dosage compensation and gene expression on the mammalian X chromosome: one plus one does not always equal two. Chromosome Res 17 (5):637–648. doi:10.1007/s10577-009-9063-9

Provinciali M, Di Stefano G, Muzzioli M, Garzetti GG, Ciavattini A, Fabris N (1995) Relationship between 17-beta-estradiol and prolactin in the regulation of natural killer cell activity during progression of endometriosis. J Endocrinol Invest 18(8):645–652

Radstake TR, Svenson M, Eijsbouts AM, van den Hoogen FH, Enevold C, van Riel PL, Bendtzen K (2009) Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. Ann Rheum Dis 68 (11):1739–1745. doi:10.1136/ard.2008.092833

Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER (2005) A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 307(5715):1625–1630. doi:10.1126/science.1106943

Roivainen M, Piirainen L, Hovi T, Virtanen I, Riikonen T, Heino J, Hyypia T (1994) Entry of coxsackievirus A9 into host cells: specific interactions with alpha v beta 3 integrin, the vitronectin receptor. Virology 203(2):357–365. doi:10.1006/viro.1994.1494

Ross MT, Grafham DV, Coffey AJ, Scherer S, McLay K, Muzny D, Platzer M, Howell GR, Burrows C, Bird CP, Frankish A, Lovell FL, Howe KL, Ashurst JL, Fulton RS, Sudbrak R, Wen G, Jones MC, Hurles ME, Andrews TD, Scott CE, Searle S, Ramser J, Whittaker A, Deadman R, Carter NP, Hunt SE, Chen R, Cree A, Gunaratne P, Havlak P, Hodgson A, Metzker ML, Richards S, Scott G, Steffen D, Sodergren E, Wheeler DA, Worley KC, Ainscough R, Ambrose KD, Ansari-Lari MA, Aradhya S, Ashwell RI, Babbage AK, Bagguley CL, Ballabio A, Banerjee R, Barker GE, Barlow KF, Barrett IP, Bates KN, Beare DM, Beasley H, Beasley O, Beck A, Bethel G, Blechschmidt K, Brady N, Bray-Allen S, Bridgeman AM, Brown AJ, Brown MJ, Bonnin D, Bruford EA, Buhay C, Burch P, Burford D, Burgess J, Burrill W, Burton J, Bye JM, Carder C, Carrel L, Chako J, Chapman JC, Chavez D, Chen E, Chen G, Chen Y, Chen Z, Chinault C, Ciccodicola A, Clark SY, Clarke G, Clee CM, Clegg S, Clerc-Blankenburg K, Clifford K, Cobley V, Cole CG, Conquer JS, Corby N, Connor RE, David R, Davies J, Davis C, Davis J, Delgado O, Deshazo D, Dhami P, Ding Y, Dinh H, Dodsworth S, Draper H, Dugan-Rocha S, Dunham A, Dunn M, Durbin KJ, Dutta I, Eades T, Ellwood M, Emery-Cohen A, Errington H, Evans KL, Faulkner L, Francis F, Frankland J, Fraser AE, Galgoczy P, Gilbert J, Gill R, Glockner G, Gregory SG, Gribble S, Griffiths C, Grocock R, Gu Y, Gwilliam R, Hamilton C, Hart EA, Hawes A, Heath PD, Heitmann K, Hennig S, Hernandez J, Hinzmann B, Ho S, Hoffs M, Howden PJ, Huckle EJ, Hume J, Hunt PJ, Hunt AR, Isherwood J, Jacob L, Johnson D, Jones S, de Jong PJ, Joseph SS, Keenan S, Kelly S, Kershaw JK, Khan Z, Kioschis P, Klages S, Knights AJ, Kosiura A, Kovar-Smith C, Laird GK, Langford C, Lawlor S, Leversha M, Lewis L, Liu W, Lloyd C, Lloyd DM, Loulseged H, Loveland JE, Lovell JD, Lozado R, Lu J, Lyne R, Ma J, Maheshwari M, Matthews LH, McDowall J, McLaren S, McMurray A, Meidl P, Meitinger T, Milne S, Miner G, Mistry SL, Morgan M, Morris S, Muller I, Mullikin JC, Nguyen N, Nordsiek G, Nyakatura G, O'Dell CN,

Okwuonu G, Palmer S, Pandian R, Parker D, Parrish J, Pasternak S, Patel D, Pearce AV, Pearson DM, Pelan SE, Perez L, Porter KM, Ramsey Y, Reichwald K, Rhodes S, Ridler KA, Schlessinger D, Schueler MG, Sehra HK, Shaw-Smith C, Shen H, Sheridan EM, Shownkeen R, Skuce CD, Smith ML, Sotheran EC, Steingruber HE, Steward CA, Storey R, Swann RM, Swarbreck D, Tabor PE, Taudien S, Taylor T, Teague B, Thomas K, Thorpe A, Timms K, Tracey A, Trevanion S, Tromans AC, d'Urso M, Verduzco D, Villasana D, Waldron L, Wall M, Wang Q, Warren J, Warry GL, Wei X, West A, Whitehead SL, Whiteley MN, Wilkinson JE, Willey DL, Williams G, Williams L, Williamson A, Williamson H, Wilming L, Woodmansey RL, Wray PW, Yen J, Zhang J, Zhou J, Zoghbi H, Zorilla S, Buck D, Reinhardt R, Poustka A, Rosenthal A, Lehrach H, Meindl A, Minx PJ, Hillier LW, Willard HF, Wilson RK, Waterston RH, Rice CM, Vaudin M, Coulson A, Nelson DL, Weinstock G, Sulston JE, Durbin R, Hubbard T, Gibbs RA, Beck S, Rogers J, Bentley DR (2005) The DNA sequence of the human X chromosome. Nature 434(7031):325–337. doi:10.1038/nature03440

Roszkowski PI, Hyc A, Malejczyk J (1993) Natural killer cell activity in patients with ovarian tumors and uterine myomas. Eur J Gynaecol Oncol 14(Suppl):114–117

Round JL, Mazmanian SK (2010) Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A 107 (27):12204–12209. doi:10.1073/pnas.0909122107

Rovati B, Mariucci S, Poma R, Tinelli C, Delfanti S, Pedrazzoli P (2013) An eight-colour flow cytometric method for the detection of reference values of lymphocyte subsets in selected healthy donors. Clin Exp Med 14:249–259. doi:10.1007/s10238-013-0239-4

Rubtsov AV, Rubtsova K, Kappler JW, Marrack P (2010) Genetic and hormonal factors in female-biased autoimmunity. Autoimmun Rev 9(7):494–498. doi:10.1016/j.autrev.2010.02.008

Sader MA, McGrath KC, Hill MD, Bradstock KF, Jimenez M, Handelsman DJ, Celermajer DS, Death AK (2005) Androgen receptor gene expression in leucocytes is hormonally regulated: implications for gender differences in disease pathogenesis. Clin Endocrinol (Oxf) 62 (1):56–63. doi:10.1111/j.1365-2265.2004.02173.x

Saha S, Tieng A, Pepeljugoski KP, Zandamn-Goddard G, Peeva E (2011) Prolactin, systemic lupus erythematosus, and autoreactive B cells: lessons learnt from murine models. Clin Rev Allergy Immunol 40(1):8–15. doi:10.1007/s12016-009-8182-6

Sandberg JK, Bhardwaj N, Nixon DF (2003) Dominant effector memory characteristics, capacity for dynamic adaptive expansion, and sex bias in the innate Valpha24 NKT cell compartment. Eur J Immunol 33(3):588–596. doi:10.1002/eji.200323707

Santiago-Raber ML, Kikuchi S, Borel P, Uematsu S, Akira S, Kotzin BL, Izui S (2008) Evidence for genes in addition to Tlr7 in the Yaa translocation linked with acceleration of systemic lupus erythematosus. J Immunol 181(2):1556–1562. doi:10.4049/jimmunol.181.2.1556

Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S (2004) Decidual and peripheral blood CD4 + CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod 10(5):347–353. doi:10.1093/molehr/gah044

Schroder J, Kahlke V, Book M, Stuber F (2000) Gender differences in sepsis: genetically determined? Shock 14(3):307–310. doi:10.1097/00024382-200014030-00011, discussion 310–303

Scotland RS, Stables MJ, Madalli S, Watson P, Gilroy DW (2011) Sex differences in resident immune cell phenotype underlie more efficient acute inflammatory responses in female mice. Blood 118(22):5918–5927. doi:10.1182/blood-2011-03-340281

Screpanti I, Santoni A, Gulino A, Herberman RB, Frati L (1987) Estrogen and antiestrogen modulation of the levels of mouse natural killer activity and large granular lymphocytes. Cell Immunol 106(2):191–202. doi:10.1016/0008-8749(87)90163-8

Seillet C, Laffont S, Tremollieres F, Rouquie N, Ribot C, Arnal JF, Douin-Echinard V, Gourdy P, Guery JC (2012) The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor alpha signaling. Blood 119(2):454–464. doi:10.1182/blood-2011-08-371831

Shames RS (2002) Gender differences in the development and function of the immune system. J Adolesc Health 30(4 Suppl):59–70. doi:10.1016/S1054-139X(01)00382-2

Shimizu I, Kohno N, Tamaki K, Shono M, Huang HW, He JH, Yao DF (2007) Female hepatology: favorable role of estrogen in chronic liver disease with hepatitis B virus infection. World J Gastroenterol 13(32):4295–4305

Sivanmaliappan TS, Sevanan M (2014) Antimicrobial Susceptibility Patterns of Pseudomonas aeruginosa from Diabetes Patients with Foot Ulcers. Int J Microbiol 2011:605195. doi:10.1155/2011/605195

Sorachi K, Kumagai S, Sugita M, Yodoi J, Imura H (1993) Enhancing effect of 17 beta-estradiol on human NK cell activity. Immunol Lett 36(1):31–35

Spach KM, Blake M, Bunn JY, McElvany B, Noubade R, Blankenhorn EP, Teuscher C (2009) Cutting edge: the Y chromosome controls the age-dependent experimental allergic encephalomyelitis sexual dimorphism in SJL/J mice. J Immunol 182(4):1789–1793. doi:10.4049/jimmunol.0803200

Spector TD, Ollier WE, Perry LA, Silman AJ (1988) Evidence for similarity in testosterone levels in haplotype identical brothers. Dis Markers 6(2):119–125

Stanberry LR, Spruance SL, Cunningham AL, Bernstein DI, Mindel A, Sacks S, Tyring S, Aoki FY, Slaoui M, Denis M, Vandepapeliere P, Dubin G (2002) Glycoprotein-D-adjuvant vaccine to prevent genital herpes. N Engl J Med 347(21):1652–1661. doi:10.1056/NEJMoa011915

Stephenson J (2000) Genital herpes vaccine shows limited promise. JAMA 284(15):1913–1914. doi:10.1001/jama.284.15.1913

Sterling TR, Vlahov D, Astemborski J, Hoover DR, Margolick JB, Quinn TC (2001) Initial plasma HIV-1 RNA levels and progression to AIDS in women and men. N Engl J Med 344 (10):720–725. doi:10.1056/NEJM200103083441003

Straub RH, Harle P, Atzeni F, Weidler C, Cutolo M, Sarzi-Puttini P (2005) Sex hormone concentrations in patients with rheumatoid arthritis are not normalized during 12 weeks of anti-tumor necrosis factor therapy. J Rheumatol 32(7):1253–1258

Subramanian S, Yates M, Vandenbark AA, Offner H (2011) Oestrogen-mediated protection of experimental autoimmune encephalomyelitis in the absence of Foxp3+ regulatory T cells implicates compensatory pathways including regulatory B cells. Immunology 132 (3):340–347. doi:10.1111/j.1365-2567.2010.03380.x

Sun SL, Horino S, Itoh-Nakadai A, Kawabe T, Asao A, Takahashi T, So T, Funayama R, Kondo M, Saitsu H, Matsumoto N, Nakayama K, Ishii N (2013) Y chromosome-linked B and NK cell deficiency in mice. J Immunol 190(12):6209–6220. doi:10.4049/jimmunol.1300303

Szekeres-Bartho J, Barakonyi A, Par G, Polgar B, Palkovics T, Szereday L (2001) Progesterone as an immunomodulatory molecule. Int Immunopharmacol 1(6):1037–1048. doi:10.1016/S1567-5769(01)00035-2

Tanriverdi F, Silveira LF, MacColl GS, Bouloux PM (2003) The hypothalamic-pituitary-gonadal axis: immune function and autoimmunity. J Endocrinol 176(3):293–304. doi:10.1677/joe.0.1760293

Tengstrand B, Carlstrom K, Hafstrom I (2002) Bioavailable testosterone in men with rheumatoid arthritis-high frequency of hypogonadism. Rheumatology (Oxford) 41(3):285–289. doi:10.1093/rheumatology/41.3.285

Teuscher C, Noubade R, Spach K, McElvany B, Bunn JY, Fillmore PD, Zachary JF, Blankenhorn EP (2006) Evidence that the Y chromosome influences autoimmune disease in male and female mice. Proc Natl Acad Sci U S A 103(21):8024–8029. doi:10.1073/pnas.0600536103

Thongngarm T, Jenkins JK, Ndebele K, McMurray RW (2003) Estrogen and progesterone modulate monocyte cell cycle progression and apoptosis. Am J Reprod Immunol 49 (3):129–138. doi:10.1034/j.1600-0897.2003.00015.x

Thuere C, Zenclussen ML, Schumacher A, Langwisch S, Schulte-Wrede U, Teles A, Paeschke S, Volk HD, Zenclussen AC (2007) Kinetics of regulatory T cells during murine pregnancy. Am J Reprod Immunol 58(6):514–523. doi:10.1111/j.1600-0897.2007.00538.x

Toder V, Nebel L, Elrad H, Blank M, Durdana A, Gleicher N (1984a) Studies of natural killer cells in pregnancy. II. The immunoregulatory effect of pregnancy substances. J Clin Lab Immunol 14(3):129–133

Toder V, Nebel L, Gleicher N (1984b) Studies of natural killer cells in pregnancy I Analysis at the single cell level. J Clin Lab Immunol 14(3):123–127

Toubiana J, Courtine E, Pene F, Viallon V, Asfar P, Daubin C, Rousseau C, Chenot C, Ouaaz F, Grimaldi D, Cariou A, Chiche JD, Mira JP (2010) IRAK1 functional genetic variant affects severity of septic shock. Crit Care Med 38(12):2287–2294. doi:10.1097/CCM.0b013e3181f9f9c7

Tsuyuguchi K, Suzuki K, Matsumoto H, Tanaka E, Amitani R, Kuze F (2001) Effect of oestrogen on Mycobacterium avium complex pulmonary infection in mice. Clin Exp Immunol 123 (3):428–434. doi:10.1046/j.1365-2249.2001.01474.x

Valliani A, Khan F, Chagani B, Khuwaja AK, Majid S, Hashmi S, Nanji K, Valliani S (2013) Factors associated with Helicobacter pylori infection, results from a developing country - Pakistan. Asian Pac J Cancer Prev 14(1):53–56. doi:10.7314/APJCP.2013.14.1.53

Valor L, Teijeiro R, Aristimuno C, Faure F, Alonso B, de Andres C, Tejera M, Lopez-Lazareno N, Fernandez-Cruz E, Sanchez-Ramon S (2011) Estradiol-dependent perforin expression by human regulatory T-cells. Eur J Clin Invest 41(4):357–364. doi:10.1111/j.1365-2362.2010.02414.x

Vernon-Roberts B (1969) The effects of steroid hormones on macrophage activity. Int Rev Cytol 25:131–159

Verthelyi D (2001) Sex hormones as immunomodulators in health and disease. Int Immunopharmacol 1(6):983–993. doi:10.1016/S1567-5769(01)00044-3

Vliagoftis H, Dimitriadou V, Boucher W, Rozniecki JJ, Correia I, Raam S, Theoharides TC (1992) Estradiol augments while tamoxifen inhibits rat mast cell secretion. Int Arch Allergy Immunol 98(4):398–409. doi:10.1159/000236217

Vultaggio A, Matucci A, Nencini F, Pratesi S, Parronchi P, Rossi O, Romagnani S, Maggi E (2010) Anti-infliximab IgE and non-IgE antibodies and induction of infusion-related severe anaphylactic reactions. Allergy 65(5):657–661. doi:10.1111/j.1398-9995.2009.02280.x

Wang C, Dehghani B, Li Y, Kaler LJ, Vandenbark AA, Offner H (2009) Oestrogen modulates experimental autoimmune encephalomyelitis and interleukin-17 production via programmed death 1. Immunology 126(3):329–335. doi:10.1111/j.1365-2567.2008.03051.x

Wickham TJ, Mathias P, Cheresh DA, Nemerow GR (1993) Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. Cell 73(2):309–319

Williams RJ, Bryan RT, Mills JN, Palma RE, Vera I, De Velasquez F, Baez E, Schmidt WE, Figueroa RE, Peters CJ, Zaki SR, Khan AS, Ksiazek TG (1997) An outbreak of hantavirus pulmonary syndrome in western Paraguay. Am J Trop Med Hyg 57(3):274–282

Wilson MA, Makova KD (2009) Evolution and survival on eutherian sex chromosomes. PLoS Genet 5(7):e1000568. doi:10.1371/journal.pgen.1000568

Woodward TL, Mienaltowski AS, Modi RR, Bennett JM, Haslam SZ (2001) Fibronectin and the alpha(5)beta(1) integrin are under developmental and ovarian steroid regulation in the normal mouse mammary gland. Endocrinology 142(7):3214–3222. doi:10.1210/endo.142.7.8273

Wunderlich F, Marinovski P, Benten WP, Schmitt-Wrede HP, Mossmann H (1991) Testosterone and other gonadal factor(s) restrict the efficacy of genes controlling resistance to Plasmodium chabaudi malaria. Parasite Immunol 13(4):357–367. doi:10.1111/j.1365-3024.1991.tb00289.x

Xia HJ, Zhang GH, Wang RR, Zheng YT (2009) The influence of age and sex on the cell counts of peripheral blood leukocyte subpopulations in Chinese rhesus macaques. Cell Mol Immunol 6 (6):433–440. doi:10.1038/cmi.2009.55

Yamamoto Y, Tomioka H, Sato K, Saito H, Yamada Y, Setogawa T (1990) Sex differences in the susceptibility of mice to infection induced by Mycobacterium intracellulare. Am Rev Respir Dis 142(2):430–433. doi:10.1164/ajrccm/142.2.430

Yamamoto Y, Saito H, Setogawa T, Tomioka H (1991) Sex differences in host resistance to Mycobacterium marinum infection in mice. Infect Immun 59(11):4089–4096

Yang F, Babak T, Shendure J, Disteche CM (2010) Global survey of escape from X inactivation by RNA-sequencing in mouse. Genome Res 20(5):614–622. doi:10.1101/gr.103200.109

Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI (2012) Human gut microbiome viewed across age and geography. Nature 486 (7402):222–227. doi:10.1038/nature11053

Zandman-Goddard G, Peeva E, Shoenfeld Y (2007) Gender and autoimmunity. Autoimmun Rev 6 (6):366–372. doi:10.1016/j.autrev.2006.10.001

Zelinkova Z, Bultman E, Vogelaar L, Bouziane C, Kuipers EJ, van der Woude CJ (2012) Sex-dimorphic adverse drug reactions to immune suppressive agents in inflammatory bowel disease. World J Gastroenterol 18(47):6967–6973. doi:10.3748/wjg.v18.i47.6967

Zhang MA, Rego D, Moshkova M, Kebir H, Chruscinski A, Nguyen H, Akkermann R, Stanczyk FZ, Prat A, Steinman L, Dunn SE (2012) Peroxisome proliferator-activated receptor (PPAR) alpha and -gamma regulate IFNgamma and IL-17A production by human T cells in a sex-specific way. Proc Natl Acad Sci U S A 109(24):9505–9510. doi:10.1073/pnas.1118458109

Chapter 2
Sex and Sex Hormones Mediate Wound Healing

Helen A. Thomason, Helen Williams, and Matthew J. Hardman

Abstract In healthy individuals cutaneous wound repair occurs through a combination of overlapping, tightly regulated phases to ensure the skin heals effectively. Disruption to these processes results in impaired healing and development of nonhealing chronic wounds. The incidence of chronic wounds is escalating as those populations most susceptible, the diabetic and elderly, continue to rise. One of the major complications associated with chronic wounds is infection. The amount and type of bacteria within a wound is linked to healing outcome. Current therapies to promote healing of chronic wounds are surprisingly limited and generally ineffective. Furthermore, the development of antibiotic-resistant bacteria and prevalence of wounds infected with recalcitrant biofilm renders current antimicrobial therapies ineffective. Treating this ever-growing problem puts financial strain on the world's health services. Thus, there is an urgent need to understand the mechanisms which result in impaired healing and to understand the differences in wound infections between males and females. Sex steroid hormones, in particular estrogens, play a pivotal role in skin maintenance of homeostasis, and research has shown that our ability to heal cutaneous wounds is modulated by host sex. A plethora of data now indicate estrogens as a primary regulator of cutaneous healing and systemic or topical oestradiol treatment promotes impaired healing. However, it is still not understood what effects estrogen treatment has on microbial profiles within a wound and how this differs between males and females. Future studies are therefore required to determine sex and gender differences in wound infections and whether hormone treatment to promote healing alters microbial profiles.

H.A. Thomason • H. Williams • M.J. Hardman (✉)
The Healing Foundation Centre, University of Manchester, A.V. Hill Building, Oxford Road, Manchester M13 9PT, UK
e-mail: Matthew.J.Hardman@manchester.ac.uk

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_2

2.1 Introduction

Wound healing involves a series of overlapping highly orchestrated processes to ensure the skin heals effectively. If these processes are disrupted, healing is hindered, resulting in a nonhealing chronic wound state. Chronic wounds, which include diabetic foot ulcers, venous leg ulcers, and pressure ulcers, are globally a significant problem. In developed countries approximately 1–2 % of the population are affected by a chronic wound during their lifetime (Gottrup 2004). Furthermore, the incidence of chronic wounds is set to rise as those most susceptible, the diabetic and elderly populations, are rapidly expanding (Sen et al. 2009). In the last decade there has been a sharp rise in the diabetic population worldwide and worryingly the rate of increase continues to rise. If obesity levels remain constant, the diabetic population is expected to double by 2030; however, if as expected obesity levels increase, the global diabetic population is expected to soar. Foot ulcers are the most common cause of hospitalization for diabetic patients and complications related to foot ulcers are the major causative factor for lower limb amputations (Chow et al. 2008). As with the diabetic population, the aging population is also rapidly expanding. Life expectancy across the world is continuing to rise and as a result the frailest populations are increasing in number at a significant rate. Elderly patients display increased susceptibility to skin injury, and furthermore, once damaged, the natural healing process is delayed leading to an increased risk of developing a chronic nonhealing wound. These most susceptible populations are putting a huge financial strain on health services worldwide. In the UK, over £3 billion of the National Health System's (NHS) expenditure is spent on treating chronic wounds and their associated problems. The cost of treating chronic wounds is set to rise as the expanding susceptible populations put more strain on world health services.

This chapter summarizes the differences in healing between males and females and discusses the expanding body of literature relating to the effects of sex hormones on healing. In addition, more recent insights into the effects of wound infection on healing are discussed, particularly in the context of how gender and sex hormones influence.

2.2 Structure of the Skin

The skin is the largest organ in our body and has a number of important functions including acting as a barrier to the external environment, mediating immune surveillance, regulating body temperature, preventing fluid loss, and providing sensory perception. The skin is composed of two layers, the outer layer, a stratified, keratinized epidermis overlying a supportive dermis. The epidermis is made up of multilayered keratinocytes held together by strong adhesion complexes including desmosomes, adherens, and tight junctions. Within the basal layer of the epidermis resides a rich supply of stem cells, and these cells give rise to

proliferative transient-amplifying cells which move progressively outwards through the epidermis where they undergo terminal differentiation, a form of programmed cell death. It is the epidermis which forms an impermeable barrier protecting the skin from its external environment. The basal layer of the epidermis is attached by hemidesmosomes to a basement membrane, a laminin and collagen IV-rich extracellular matrix (ECM). This basement membrane separates the epidermis from the underlying dermis. The underlying supportive dermis is composed of fibroblasts embedded in a collagen-rich ECM and supplies nutrients to the avascular epidermis while cushioning the underlying tissues from trauma. The extracellular matrix in the adult dermis is complex, composed of collagen fibers (for strength), elastic fibers (for resilience), and glycosaminoglycans (for hydration). The dermis is divided into two layers, papillary and reticular. The papillary dermis lies immediately beneath the epidermis and is composed of loosely packed collagen fibers, whereas the reticular dermis which resides beneath the papillary dermis is composed of tightly packed collagen fibers and elastic fibers. Within the dermis reside many of the skin appendages such as sweat glands, hair follicles, lymph nodes, touch receptors, and vasculature system (Wysocki 1999).

2.3 Sex Differences in Skin Structure and Function

There are a number of sex differences in the structure of the skin. Male human skin is thicker with greater collagen density than female skin (Seidenari et al. 1994), although females have thicker subcutaneous tissue (Sjostrom et al. 1972). In both male and female skin, there are a number of morphological changes which occur during aging, including a decline in dermal thickness, a decrease in absolute cell numbers, and flattening of the dermo-epidermal junction (Ashcroft et al. 2002). However, after menopause, when estrogen and progesterone levels drop rapidly, the skin alters dramatically; the epidermis and dermis thin, and there is a decrease in collagen content (Brincat et al. 1987) and a reduction in elasticity (Sumino et al. 2004); and the skin becomes dryer and more fragile and bruises easily (Leveque et al. 1984; Brincat et al. 1985; Ya-Xian et al. 1999). Topical or systemic administration of estrogens is able to reverse many of the adverse effects of skin aging (Brincat et al. 1985; Punnonen 1971; Shuster et al. 1975; Varila et al. 1995). Systemic hormone replacement therapy significantly increases the collagen content of the skin (Brincat et al. 1987; Sauerbronn et al. 2000), increases epidermal thickness (Hall and Phillips 2005), as well as improves skin hydration (Hall and Phillips 2005). At a cellular level, estrogens stimulate keratinocyte proliferation, inhibit apoptosis, and dampen skin protease levels (Brincat 2000). Topical estrogen treatment maintains skin thickness (Shah and Maibach 2001) and increases skin moisture content and barrier function. In addition, topical 17beta-estradiol treatment induces dermal production of collagens I and III (Son et al. 2005), while estradiol treatment increases the number and thickness of dermal elastin fibers and improves their orientation (Punnonen et al. 1987). Furthermore, the macroscopic

appearance is improved with a significant reduction in fine wrinkles (Brincat et al. 1985).

Historic evidence indicates that sex steroids in females, primarily estrogens, have an important function in the treatment of skin disorders. For example, topical application of "follicular hormone," now known to be 17β-estradiol, was shown to locally improve acne and eczema (Loeser 1937). The symptoms of skin disorders such as psoriasis improve during pregnancy when estrogen levels are high (Dunna and Finlay 1989; Boyd et al. 1996) and oral contraceptive pills are often prescribed for the treatment of severe acne (Arowojolu et al. 2009). Furthermore, an increasing amount of data now indicates that estrogens are key modulators of wound repair, with estrogenic treatment promoting healing.

2.4 Overview of Acute Wound Healing

If injured, it is essential that the skin repairs itself effectively and rapidly to ensure the functions of the skin are not compromised. Wound healing involves a complex series of tightly regulated, overlapping processes. Upon injury, blood vessels constrict to prevent excessive blood loss and a fibrin clot forms which acts as a temporary barrier (Clark 1990). Degranulation of platelets embedded within the clot releases a cocktail of proinflammatory cytokines and growth factors which act as chemotactic cues to recruit circulating inflammatory cells. These cytokines and growth factors later stimulate proliferation of keratinocytes and fibroblasts to promote re-epithelialization and wound closure (Anitua et al. 2004). Endothelial cells lining the blood vessels undergo specific changes allowing macrophages and neutrophils to adhere, roll, and undergo diapedesis to exit blood vessels and enter the wounded tissue (Schober and Weber 2005). The infiltration of inflammatory cells into the wounded tissue heightens the inflammatory response by releasing additional cytokines and growth factors (Gillitzer and Goebeler 2001). Neutrophils are the first inflammatory cells to arrive at the wound. Their main function is to clear foreign debris and microbes which have entered the wound, by releasing lysosomal enzymes and proteases that facilitate microbial destruction and removal of damaged matrix. Following neutrophil infiltration, monocytes enter the wound and differentiate into macrophages. Here, macrophages adhere to the ECM and once activated secrete a range of matrix metalloproteinases (MMPs) that degrade and remodel damaged ECM proteins in addition to releasing their own profile of growth factors and cytokines to modulate the established inflammatory response. Macrophages remain within the wound after neutrophil infiltration has subsided to phagocytose any remaining pathogenic material, cell debris, and damaged ECM (Martin et al. 1988; Fujiwara and Kobayashi 2005).

The release of cytokines and growth factors from platelets, neutrophils, and macrophages initiates a burst of proliferation within the wound. Fibroblasts proliferate and migrate into the newly formed granulation tissue where they secrete a provisional collagen III-rich matrix. Concurrently, to degrade and remodel the

existing ECM, fibroblasts secrete a mixture of MMPs, including collagenases, stromelysins, and gelatinases (Salo et al. 1994). A subset of fibroblasts within the wound, when stimulated by transforming growth factor beta 1 (TGF-β1), differentiate into myofibroblasts, a specialized subset of fibroblasts which express smooth muscle actin capable of generating a contractile force to close the wound through contraction (Desmoulière et al. 1993; Vaughan et al. 2000; Hinz 2007).

Stimulated by a plethora of growth factors secreted by platelets, inflammatory cells, fibroblasts, and keratinocytes, keratinocytes at the wound margin proliferate and migrate through the granulation tissue to re-epithelialize the wound (Barrandon and Green 1987; Décline et al. 2003; Iwamoto and Mekada 2000; Werner et al. 1992; Raja et al. 2007; Haase et al. 2003). To aid migration, keratinocytes release tissue-type-specific plasminogen activator (tPA) and urokinase plasminogen activator (uPA) that convert plasminogen in the fibrin-rich clot to the enzyme plasmin, which digests a pathway for the migrating cells (Grøndahl-Hansen et al. 1988; Rømer et al. 1994; Ossowski and Aguirre-Ghiso 2000). Desmosomal adhesion between keratinocytes is modified (Thomason et al. 2012) and hemidesmosomes are downregulated to facilitate migration (Krawczyk and Wilgram 1973; Litjens et al. 2006). Stem cells within the basal layer of the epidermis and hair follicles repopulate the epidermis as keratinocytes migrate. Once the epithelial edges have met, migration ceases and keratinocytes differentiate into a newly stratified epidermis. In contrast to normal skin, newly formed tissue lacks skin appendages such as hair follicles and sweat glands, and the neo-epidermis lacks undulations and rete ridges observed in normal skin. While the wound is undergoing re-epithelialization, growth factors such as fibroblast growth factor 2 (FGF2), vascular endothelial growth factor (VEGF), tumor necrosis factor alpha (TNF-α), and TGF-α stimulate proliferation and migration of endothelial cells to form a new vascular system (Li et al. 2003). Upregulation of $\alpha_v\beta_3$ integrins at the tips of developing capillaries is vital for angiogenesis to occur (Brooks et al. 1994).

The final stage of wound healing, the remodeling phase, is by far the longest process. In this phase, collagen fibers are degraded, synthesized, reorganized, and stabilized ultimately resulting in the formation of a connective tissue scar. Central to the remodeling phase is the proteolytic activity of MMPs (Moses et al. 1996). Newly formed collagen is laid down in a pattern very different from that of unwounded skin, with collagen fibers deposited in characteristic parallel arrays. As this phase progresses, the collagen content of the wound decreases and the tensile strength increases through cross-linking of collagen fibers; however, the tensile strength of unwounded skin is never achieved.

2.5 Pathological Wound Healing

When healing fails to proceed in this orderly and timely manner, the wound is classed as "chronic" (Lazarus et al. 1994) (Fig. 2.1). The three most common types of chronic wounds are diabetic foot ulcers, venous leg ulcers, and pressure ulcers. Chronic wounds normally present as a comorbid condition with other underlying pathologies such as nutrient deficiency, alcoholism, advancing age, and chronic diseases such as diabetes and renal disease. Local factors also influence the development of chronic wounds including, neuropathy, ischemia, tissue maceration, and infection.

The development of a chronic wound involves complex multifactorial molecular mechanisms that prevent healing. At the cellular level chronic wounds show excessive inflammation, with increased numbers of macrophage and neutrophils. The destructive nature of these cell types on the ECM, endothelial, fibroblast, and smooth muscle cells contributes to the pathology of chronic wounds (Loots et al. 1998; Diegelmann 2003). The ECM within chronic wounds diminishes through reduced synthesis and increased degradation (Blakytny and Jude 2009). Reduced growth factors which stimulate the production of collagen and elastin from fibroblasts and an increase in MMPs, such as MMP2 and MMP9, contribute to the reduced ECM (Blakytny et al. 2000; Galkowska et al. 2006; Yager et al. 1996;

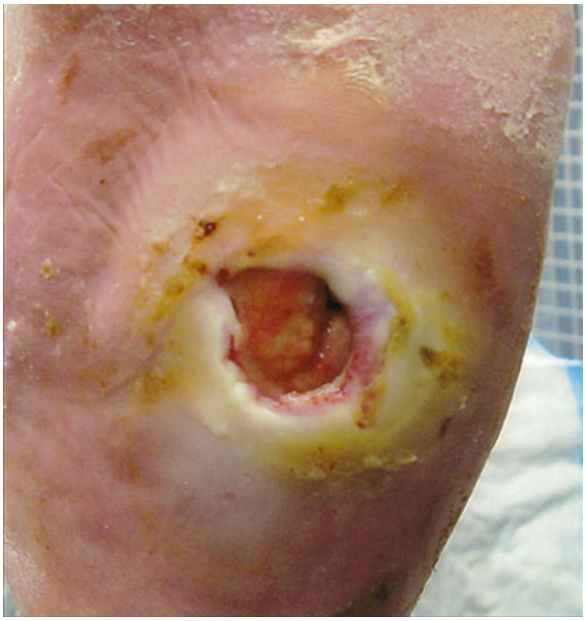

Fig. 2.1 Macroscopic image of a chronic wound. When the natural process of acute healing is disrupted, a chronic nonhealing wound state ensues. The epidermis breaks down leaving the wound at risk of infection

Lobmann et al. 2002). Re-epithelialization in chronic wounds is often delayed. Although keratinocytes within a chronic wound are highly proliferative, they fail to migrate across the wound bed and express an abnormal keratin profile (Andriessen et al. 1995). Growth factors which stimulate keratinocyte migration are downregulated in chronic wounds (Blakytny and Jude 2009). Excessive proteolysis of ECM components next to the wound edge also inhibits migration as keratinocytes deposit and require basement membrane components in order to migrate (Pastar et al. 2008). Angiogenesis within a wound is essential to supply a new vasculature system to the damaged tissue providing nutrient and oxygen for effective tissue repair. Diabetic foot ulcers and venous leg ulcers display an abnormal microcirculation (Luetolf et al. 1993; Schramm et al. 2006). A number of factors are thought to contribute to abnormal angiogenesis including reduced levels of growth factors such as platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), and IL-8, which stimulate endothelial cell proliferation and migration (Blakytny et al. 2000; Galkowska et al. 2006). In addition, the degradation of the ECM, an essential scaffold for endothelial cell migration, also hinders new blood vessel formation (Blakytny and Jude 2009; Rundhaug 2005).

2.6 Sex Differences in Wound Healing

Age negatively influences the phases of tissue repair, where the natural progression of acute wound healing is disrupted after skin injury and a chronic nonhealing wound state ensues (Ashcroft et al. 1998). Yet research has indicated that specific changes in the healing response are also modulated by host sex. This is not surprising given that sex steroid hormones, in particular estrogens, play such a pivotal role in skin maintenance of homeostasis. Being male is considered a risk factor for abnormal wound healing in the elderly. Clinical studies have shown that men have an altered inflammatory response and take longer to heal acute dermal wounds compared to women, thus suggesting a female advantage in healing rates (Herrick et al. 1997; Ashcroft et al. 1999). Conversely, when observing oral mucosal wounds, a male advantage in healing rates has been reported (Engeland et al. 2009). In addition, mucosal wound healing following oral surgical procedures is also associated with greater complications and longer recovery times in women (Conrad et al. 1999; Phillips et al. 2003; Benediktsdottir et al. 2004; Adeyemo et al. 2006). This implies that the sex differences in healing rate are potentially tissue dependent (Engeland et al. 2009). In humans, the production of dehydroepiandrosterone sulfate (DHEAS), the common sex hormone precursor, and its metabolite dehydroepiandrosterone (DHEA) declines significantly with age (Labrie et al. 1997). DHEA is locally converted into estrogens and androgens. When considering cutaneous healing, reduced systemic DHEA levels positively correlate with an increased risk of developing a chronic (nonhealing) venous leg ulcer (Mills et al. 2005). In female mice, systemic DHEA treatment completely reverses the

delayed wound healing phenotype exhibited by mice lacking systemic estrogen and local DHEA treatment reverses impaired healing in aged male mice (Mills et al. 2005). DHEA administration may therefore act as an effective therapy for treatment of delayed healing wounds in older people.

Our microarray analysis of wounds from young and elderly men found that 78 % of genes differentially expressed between the two groups were estrogen regulated, while only 3 % were age associated, strongly implicating reduced estrogen, and not known gerontogenes, as the primary regulator of delayed healing in aged subjects (Hardman and Ashcroft 2008). Thus, it is not surprising that elderly females, with dramatically lower levels of estradiol, have impaired healing responses (Ashcroft et al. 1997a, 1999). An abrupt reduction in estradiol levels in these women corresponds with a decrease in the stimulation of cutaneous estrogen receptors (ERs), along with downstream impaired cytokine signal transduction, an altered protein balance, and destructive levels of inflammation (Fimmel and Zouboulis 2005). When applied topically or given systemically, 17β-estradiol can reverse this age-related impairment in healing in elderly females and is associated with a dampening of excessive inflammation, preventing disproportionate elastase production, enhancing matrix deposition, and accelerating re-epithelialization (Ashcroft and Ashworth 2003; Mills et al. 2005) (Fig. 2.2). Strikingly, hormone replacement therapy has been shown to protect postmenopausal women from developing venous leg ulcers or pressure ulcers (Margolis et al. 2002).

Elderly males respond substantially less to estrogen treatment than females of the same age (Fimmel and Zouboulis 2005), suggesting there are other factors involved beyond the effects of reduced estrogens, namely, the antagonistic role of the male sex hormone, testosterone. It is reported that comparatively high testosterone levels in elderly males are positively correlated with a delay in wound repair (Fimmel and Zouboulis 2005). High testosterone levels are associated with increased susceptibility to infection and impaired wound healing after trauma and hemorrhage, along with increases in proinflammatory cytokine expression and local inflammation (Ashcroft et al. 2002; Fimmel and Zouboulis 2005). Additional reports indicate that being male is a risk factor for impaired healing of chronic venous leg ulcers (Taylor et al. 2002), which are characterized by unresolved inflammation and excessive proteolysis (Wysocki and Staiano-Cioco 1993). Unlike the rapid decline in estradiol levels after the menopause in females, circulating testosterone gradually declines in males during aging which may explain why elderly men reportedly heal more slowly than elderly women even following estrogen treatment (Ashcroft et al. 1999).

Animal models have provided key insight into the roles of estrogens and androgens in wound healing. Surgical removal of ovaries in adult mice and rats has been shown to significantly delay healing, increase inflammatory cell recruitment, delay re-epithelialization, and reduce collagen deposition. This delay in healing can be reversed by topical or systemic administration of estradiol or phytoestrogens, including genistein at a level similar to an infant consuming soy-based infant formula (Ashcroft et al. 1997a; Ashcroft and Ashworth 2003;

Emmerson et al. 2010; Hardman and Ashcroft 2008). Estrogen acts as a keratinocyte mitogen and is able to promote closure of confluent keratinocyte monolayer scratch wounds in vitro and accelerate re-epithelialization in ovariectomized mice (Emmerson et al. 2009, 2010; Campbell et al. 2010; Hardman and Ashcroft 2008). Recently, studies have started to elucidate the mechanisms by which estrogen promotes healing. Estrogens primarily signal through two nuclear hormone receptors, estrogen receptor alpha and beta (ERα and ERβ), which are differentially expressed throughout mammalian tissues. Both isoforms are expressed in the skin, although there is some contention over relative levels of key skin cell types, primarily keratinocytes, fibroblasts, and immune cells. Using isoform-specific knockout mice, the beneficial effects of estradiol on healing are shown to be mediated through ERβ, whereas signaling through ERα alone has a detrimental effect on healing (Campbell et al. 2010). Strikingly, PPT (4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl) and DPN (2,3-bis(4-hydroxyphenyl)-propionitrile), agonists to ERα or ERβ, respectively, both dampen the heightened inflammation observed in delayed healing ovariectomized adult mice. However, DPN alone promoted healing, suggesting a complex situation where ERβ is predominant in estrogen beneficial effects on healing but ERα-mediated anti-inflammatory actions could be important in certain contexts (Campbell et al. 2010). In contrast to removing estrogen, eliminating testosterone through castration of young male mice and rats accelerates cutaneous healing. This is associated with increased matrix deposition and a dampened inflammatory response, in which macrophage and neutrophil influx are reduced and the proinflammatory cytokines IL-6 and TNF-α are downregulated (Ashcroft and

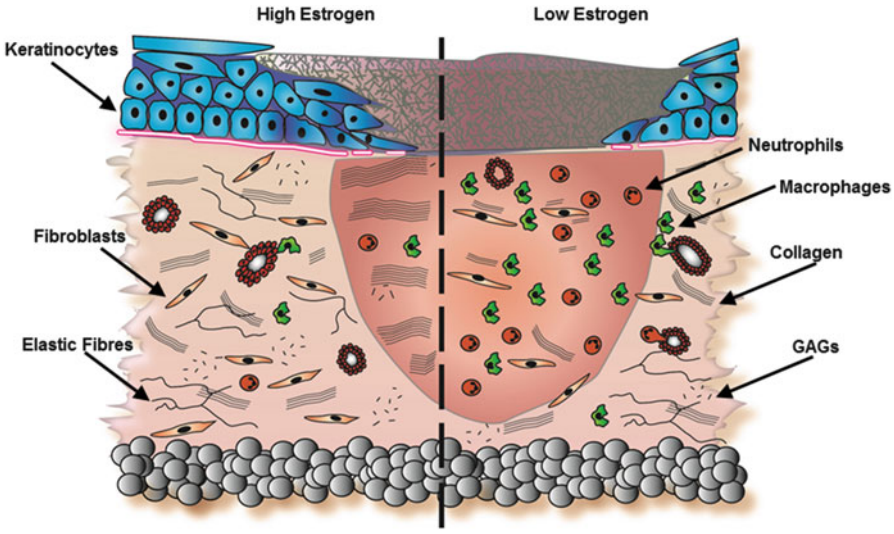

Fig. 2.2 Estrogen treatment promotes healing. Topical or systemic estradiol treatment increases keratinocyte proliferation and migration, stimulating re-epithelialization. In addition, estradiol dampens excessive inflammation and stimulates fibroblast proliferation and migration increasing extracellular matrix deposition

Mills 2002; Mills et al. 2005). In addition, in hairless mice, castration enhances proliferation of hair follicles and also increases cutaneous wound healing. This has been shown through a direct effect of testosterone on murine macrophage TNF-α production via the androgen receptor, in parallel to downregulation of TNF-α following castration (Ashcroft and Mills 2002). In humans, accumulating evidence suggests significant gender differences following major infection, and several studies indicate that immune functions in males are depressed following trauma hemorrhage yet are enhanced in females. Furthermore, in a rodent model, when subjected to artificially induced trauma (burns) and haemorrhagic shock, castrated male mice have an increased survival rate associated with immune recovery, identified through markedly reduced levels of systemic IL-6 and TNF-α compared with intact male mice (Wichmann et al. 1996). Conversely, adult female mice with intact ovaries showed enhanced immune function, but when administered testosterone led to a significant depression of cell-mediated immunity (decreased splenocyte proliferation and proinflammatory cytokine IL-2 and IL-3 secretion after traumatic injury; laparotomy and hemorrhagic shock) comparable to that seen in intact male mice (Angele et al. 1998). Thus, high testosterone levels appear to be responsible for immune depression in males after trauma hemorrhage, and in relation to cutaneous healing, such agents that block testosterone receptors may therefore be helpful in improving wound repair. Overall, androgens promote local inflammatory responses and lengthen healing time, whereas estrogens exhibit anti-inflammatory effects and shorten healing times (Ashcroft and Ashworth 2003; Jarefors et al. 2006; Gilliver et al. 2007).

2.7 Wound Infection

Human skin is colonized by numerous commensal or mutualistic microbial populations, which coexist peacefully with the host, with no or some benefit, respectively. Recent technological advances in molecular profiling and subsequent research efforts, including the US National Institutes of Health-funded Human Microbiome Project, have allowed comprehensive investigation of the diversity of healthy human skin microbiome under homeostatic conditions (Peterson et al. 2009). These studies report that bacterial diversity in males and females depends on the topographical location on the body and that the observed temporal variability is dependent on the site sampled (Grice et al. 2008, 2009). This microbial diversity is thought to be determined through a variety of factors including transmission of nonresident microbes, genetic predisposition, lifestyle, and environmental characteristics (Ehlers and Kaufmann 2010; Rosenthal et al. 2011). This natural microbiome prevents pathogenic bacteria colonizing the skin by competing for space and nutrients or secreting chemicals which inhibit their growth. Little is known however about the role of sex steroid hormones in manipulating the skin microbiome. It has been found that host gender shapes the skin environment,

thereby influencing the colonizing bacterial profile. Women have been shown to have significantly greater bacterial diversity on their hands in comparison to men (Fierer et al. 2008). A recent study observed sex differences in the microbiota of the stratum corneum of normal buttock skin before and after tape stripping (to create a superficial wound with erythema and transepidermal water loss; Zeeuwen et al. 2012). Possible explanations for these sex-related differences may include hormone balance, sweat or sebum production, or skin pH variation. Differences in the microbiota composition of the skin might also reflect gender differences associated with host behavior or a combination of intrinsic and extrinsic factors.

The human immune system tolerates commensal or mutualistic microbial populations without activating an immune response. When the skin is compromised, the warm, moist, and highly nutritive environment of the subcutaneous tissue allows microbial populations to colonize the wound. This may occur through resident microbiota becoming pathogenic or through new pathogenic species colonizing the wound (Roth and James 1988). Thus, wounds which are slow or fail to heal become increasingly susceptible to infection. Wounds are defined as infected when pathogen colonization exceeds 10^6 organisms per gram of tissue and when they show clinical signs of infection, principally heat, edema, and odor (Robson 1997). Wound bioburden, which takes into account the microbial load and diversity within a wound, is linked to healing outcome (Gardner and Frantz 2008). The presence of four or more microbial species within a wound is associated with poor healing (Trengove et al. 1996).

An additional complication associated with wound infections is the infection which often manifests as a biofilm. Biofilms differ in a number of ways from "free floating" planktonic bacteria; within a biofilm, bacteria aggregate and encase themselves in a self-secreted extracellular polymeric substance (EPS). Bacteria within a biofilm show a more sessile growth pattern, reduced metabolic rate, altered nutritional requirement, and gene transcription when compared to planktonic bacteria (Joo and Otto 2012). Furthermore, mature biofilms develop specialized structures including channels to transfer water, nutrients, and waste products. The growth of the biofilm is guided by self-secreted intracellular signaling molecules to guide growth in response to nutrient availability, a phenomenon known as quorum sensing. These differences provide the microbes with the optimal environment allowing microbes within a biofilm to avoid the host immune response and display up to 1,000 times greater resistance to antimicrobial treatments when compared to their planktonic counterparts.

Microbiological findings in chronic wounds vary depending on the sampling and diagnostic methods, but generally speaking the most common bacterial findings in human wounds are also present on normal skin and include *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus faecalis*, *Proteus species*, anaerobic bacteria, and *Pseudomonas aeruginosa* (Gjødsbøl et al. 2006). The detrimental effect of microbial infection on chronic nonhealing wounds has long been recognized as a principle aspect of wound management. In addition, wound infections are the most expensive complications following a surgical procedure and are thought to drive hospital-acquired infections (Sen et al. 2009).

2.8 Effects of Sex and Sex Hormones on Wound Infection

Accumulating evidence supports a role for sex and gender differences in the incidence of and outcome following major infection and infectious diseases (Klein 2000). In response to trauma, hemorrhage, and sepsis, women have been found to have significant survival advantages over men (see Chap. 9) (George et al. 2003; Oberholzer et al. 2000), and following surgical sepsis women have a marked reduction in hospital mortality, with a rate of 26 % in women versus 70 % in men (Schroder et al. 1998). These findings suggest in general males are at a greater risk for post-injury infections, in line with their increased susceptibility to infectious diseases such as cutaneous leishmaniases, pulmonary tuberculosis, lepromatous leprosy, typhoid fever, leptospirosis, meningococcal meningitis, and hepatitis A (Guerra-Silveira and Abad-Franch 2013). Interestingly, this male bias in general infectious disease susceptibility is also apparent in infancy, when sex steroid hormone levels transiently rise. The physiology underlying these gender-specific effects remains largely unclear, with particularly poor understanding of sex steroid hormone regulation of relevant aspects of host defense.

It is well established that sex steroid hormones are known to have immunomodulatory properties, and importantly a well-documented dichotomy exists in the immune response to injury between the sexes. This is thought to be the result of differences in inflammation, specifically immune cell activation, infiltration, and cytokine production during and following injury (Ashcroft and Mills 2002; Gilliver et al. 2007). Estrogen and androgen receptors are present on dendritic cells (Kovats and Carreras 2008), macrophages (Lai et al. 2009), lymphocytes (Marriott and Huet-Hudson 2006), neutrophils (Pergola et al. 2008), and mast cells (Narita et al. 2007). Interestingly, estrogen improves neutrophil phagocytic ability, suggesting that higher levels of estrogen can aid clearance of infection through increased neutrophil function (Magnusson and Einarsson 1990). Estrogens and androgens have complex interactions with immune cell function, and it is important to note that they can either positively and/or negatively regulate the immune response by aiding resolution or by compounding morbidity and mortality depending on which immune responses are being observed (Bird et al. 2008). Estrogens are generally thought to enhance the humoral immune response (Nikolaevich et al. 1991), while androgens act to suppress both cell-mediated and humoral responses (Kocar et al. 2000).

2.9 Summary

The structure and function of our skin alters with age as systemic estrogen levels drop. These changes result in a more fragile skin which takes longer to heal. Chronic wounds which fail to heal are a significant problem worldwide and as the elderly population continues to grow the incidence of chronic nonhealing wounds is

set to rise. One of the major complications associated with chronic wounds is infection. The amount and type of bacteria within a wound is linked to healing outcome; however, little is known about the differences in wound infections between males and females. Estrogen treatment has been shown to promote healing in vitro, in vivo, and in the clinic. However, it is still not understood what effects estrogen treatment has on microbial profiles within a wound and how this differs between males and females. Thus, future studies are needed to determine sex and gender differences in wound infections and whether hormone treatment to promote healing alters microbial profiles.

References

Adeyemo WL, Ladeinde AL, Ogunlewe MO (2006) Clinical evaluation of post-extraction site wound healing. J Contemp Dent Pract 7:40–49

Andriessen MP, van Bergen BH, Spruijt KI, Go IH, Schalkwijk J, van de Kerkhof PC (1995) Epidermal proliferation is not impaired in chronic venous ulcers. Acta Derm Venereol 75:459–462

Angele MK, Ayala A, Cioffi WG, Bland KI, Chaudry IH (1998) Testosterone: the culprit for producing splenocyte immune depression after trauma hemorrhage. Am J Physiol 274:C1530–C1536

Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT (2004) Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost 91:4–15

Arowojolu AO, Gallo MF, Lopez LM, Grimes DA, Garner SE (2009) Combined oral contraceptive pills for treatment of acne. Cochrane Database Syst Rev 8:CD004425

Ashcroft GS, Ashworth JJ (2003) Potential role of estrogens in wound healing. Am J Clin Dermatol 4:737–743

Ashcroft GS, Mills SJ (2002) Androgen receptor-mediated inhibition of cutaneous wound healing. J Clin Invest 110:615–624

Ashcroft GS, Dodsworth J, Boxtel E, Tarnuzzer R, Horan MA, Schultz GS, Ferguson MWJ (1997a) Estrogen accelerates cutaneous wound healing associated with an increase in TGF-b1 levels. Nat Med 3:1209–1215

Ashcroft GS, Horan MA, Ferguson MW (1998) Aging alters the inflammatory and endothelial cell adhesion molecule profiles during human cutaneous wound healing. Lab Invest 78:47–58

Ashcroft GS, Greenwell-Wild T, Horan MA, Wahl SM, Ferguson MW (1999) Topical estrogen accelerates cutaneous wound healing in aged humans associated with an altered inflammatory response. Am J Pathol 155:1137–1146

Ashcroft GS, Mills SJ, Ashworth JJ (2002) Ageing and wound healing. Biogerontology 3:337–345

Barrandon Y, Green H (1987) Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor-alpha and epidermal growth factor. Cell 50:1131–1137

Benediktsdottir IS, Wenzel A, Petersen JK, Hintze H (2004) Mandibular third molar removal: risk indicators for extended operation time, postoperative pain, and complications. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 97:438–446

Bird MD, Karavitis J, Kovacs EJ (2008) Sex differences and estrogen modulation of the cellular immune response after injury. Cell Immunol 252:57–67

Blakytny R, Jude EB (2009) Altered molecular mechanisms of diabetic foot ulcers. Int J Low Extrem Wounds 8:95–104

Blakytny R, Jude EB, Martin Gibson J, Boulton AJ, Ferguson MW (2000) Lack of insulin-like growth factor 1 (IGF1) in the basal keratinocyte layer of diabetic skin and diabetic foot ulcers. J Pathol 190:589–594

Boyd AS, Morris LF, Phillips CM, Menter MA (1996) Psoriasis and pregnancy: hormone and immune system interaction. Int J Dermatol 35:169–172

Brincat MP (2000) Hormone replacement therapy and the skin. Maturitas 35:107–117

Brincat M, Moniz CJ, Studd JW, Darby A, Magos A, Emburey G, Versi E (1985) Long-term effects of the menopause and sex hormones on skin thickness. Br J Obstet Gynaecol 92:256–259

Brincat M, Kabalan S, Studd JW, Moniz CF, de Trafford J, Montgomery J (1987) A study of the decrease of skin collagen content, skin thickness, and bone mass in the postmenopausal woman. Obstet Gynecol 70:840–845

Brooks PC, Clark RA, Cheresh DA (1994) Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science 264:569–571

Campbell L, Emmerson E, Davies F, Gilliver SC, Krust A, Chambon P, Ashcroft GS, Hardman MJ (2010) Estrogen promotes cutaneous wound healing via estrogen receptor beta independent of its antiinflammatory activities. J Exp Med 207:1825–1833

Chow I, Lemos EV, Einarson TR (2008) Management and prevention of diabetic foot ulcers and infections: a health economic review. Pharmacoeconomics 26:1019–1035

Clark RA (1990) Fibronectin matrix deposition and fibronectin receptor expression in healing and normal skin. J Invest Dermatol 94(Suppl 6):128S–134S

Conrad SM, Blakey GH, Shugars DA, Marciani RD, Phillips C, White RP Jr (1999) Patients' perception of recovery after third molar surgery. J Oral Maxillofac Surg 57:1288–1294

Décline F, Okamoto O, Mallein-Gerin F, Helbert B, Bernaud J, Rigal D, Rousselle P (2003) Keratinocyte motility induced by TGF-beta1 is accompanied by dramatic changes in cellular interactions with laminin 5. Cell Motil Cytoskeleton 54:64–80

Desmoulière A, Geinoz A, Gabbiani F, Gabbiani G (1993) Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol 122:103–111

Diegelmann RF (2003) Excessive neutrophils characterize chronic pressure ulcers. Wound Repair Regen 11:490–495

Dunna SF, Finlay AY (1989) Psoriasis: improvement during and worsening after pregnancy. Br J Dermatol 120:584

Ehlers S, Kaufmann SH (2010) Infection, inflammation, and chronic diseases: consequences of a modern lifestyle. Trends Immunol 31:184–190

Emmerson E, Campbell L, Ashcroft GS, Hardman MJ (2009) Unique and synergistic roles for 17beta-estradiol and macrophage migration inhibitory factor during cutaneous wound closure are cell type specific. Endocrinology 150:2749–2757

Emmerson E, Campbell L, Ashcroft GS, Hardman MJ (2010) The phytoestrogen genistein promotes wound healing by multiple independent mechanisms. Mol Cell Endocrinol 321:184–193

Engeland CG, Sabzehei B, Marucha PT (2009) Sex hormones and mucosal wound healing. Brain Behav Immun 23:629–635

Fierer N, Hamady M, Lauber CL, Knight R (2008) The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc Natl Acad Sci U S A 105:17994–17999

Fimmel S, Zouboulis CC (2005) Influence of physiological androgen levels on wound healing and immune status in men. Aging Male 8:166–174

Fujiwara N, Kobayashi K (2005) Macrophages in inflammation. Curr Drug Targets Inflamm Allergy 4:281–286

Galkowska H, Wojewodzka U, Olszewski WL (2006) Chemokines, cytokines, and growth factors in keratinocytes and dermal endothelial cells in the margin of chronic diabetic foot ulcers. Wound Repair Regen 14:558–565

Gardner SE, Frantz RA (2008) Wound bioburden and infection-related complications in diabetic foot ulcers. Biol Res Nurs 10:44–53

George RL, McGwin G Jr, Metzger J, Chaudry IH, Rue LW (2003) The association between gender and mortality among trauma patients as modified by age. J Trauma 54:464–471
Gillitzer R, Goebeler M (2001) Chemokines in cutaneous wound healing. J Leukoc Biol 69:513–521
Gilliver SC, Ashworth JJ, Ashcroft GS (2007) The hormonal regulation of cutaneous wound healing. Clin Dermatol 25:56–62
Gjødsbøl K, Christensen JJ, Karlsmark T, Jørgensen B, Klein BM, Krogfelt KA (2006) Multiple bacterial species reside in chronic wounds: a longitudinal study. Int Wound J 3:225–231
Gottrup FA (2004) Specialized wound-healing center concept: importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. Am J Surg 187:38S–43S
Grice EA, Kong HH, Renaud G, Young AC, Bouffard GG, Blakesley RW, Wolfsberg TG, Turner ML, Segre JA (2008) A diversity profile of the human skin microbiota. Genome Res 18:1043–1050
Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, Bouffard GG, Blakesley RW, Murray PR, Green ED, Turner ML, Segre JA (2009) Topographical and temporal diversity of the human skin microbiome. Science 324:1190–1192
Grøndahl-Hansen J, Lund LR, Ralfkiaer E, Ottevanger V, Danø K (1988) Urokinase- and tissue-type plasminogen activators in keratinocytes during wound reepithelialization in vivo. J Invest Dermatol 90:790–795
Guerra-Silveira F, Abad-Franch F (2013) Sex bias in infectious disease epidemiology: patterns and processes. PLoS One 8:e62390
Haase I, Evans R, Pofahl R, Watt FM (2003) Regulation of keratinocyte shape, migration and wound epithelialization by IGF-1- and EGF-dependent signalling pathways. J Cell Sci 116:3227–3238
Hall G, Phillips TJ (2005) Estrogen and skin: the effects of estrogen, menopause, and hormone replacement therapy on the skin. J Am Acad Dermatol 53:555–568
Hardman MJ, Ashcroft GS (2008) Estrogen, not intrinsic aging, is the major regulator of delayed human wound healing in the elderly. Genome Biol 9:R80
Herrick SE, Ashcroft G, Ireland G, Horan M, McCollum C, Ferguson M (1997) Up-regulation of elastase in acute wounds of healthy aged humans and chronic venous leg ulcers is associated with matrix degradation. Lab Invest 77:281–288
Hinz B (2007) Formation and function of the myofibroblast during tissue repair. J Invest Dermatol 127:526–537
Iwamoto R, Mekada E (2000) Heparin-binding EGF-like growth factor: a juxtacrine growth factor. Cytokine Growth Factor Rev 11:335–344
Jarefors S, Bennet L, You E, Forsberg P, Ekerfelt C, Berglund J, Ernerudh J (2006) Lyme borreliosis reinfection: might it be explained by gender difference in immune response? Immunology 118:224–232
Joo HS, Otto M (2012) Molecular basis of in vivo biofilm formation by bacterial pathogens. Chem Biol 19:1503–1513
Klein SL (2000) The effects of hormones on sex differences in infection: from genes to behavior. Neurosci Biobehav Rev 24:627–638
Kocar IH, Yesilova Z, Ozata M, Turan M, Sengul A, Ozdemir I (2000) The effect of testosterone replacement treatment on immunological features of patients with Klinefelter's syndrome. Clin Exp Immunol 121:448–452
Kovats S, Carreras E (2008) Regulation of dendritic cell differentiation and function by estrogen receptor ligands. Cell Immunol 252:81–90
Krawczyk WS, Wilgram GF (1973) Hemidesmosome and desmosome morphogenesis during epidermal wound healing. J Ultrastruct Res 45:93–101
Labrie F, Bélanger A, Cusan L, Gomez JL, Candas B (1997) Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. J Clin Endocrinol Metab 82:2396–2402

Lai JJ, Lai KP, Chuang KH, Yu PI-C, Lin WJ, Chang C (2009) Monocyte/macrophage androgen receptor suppresses cutaneous wound healing in mice by enhancing local TNF-α expression. J Clin Invest 119:3739–3751

Lazarus GS, Cooper DM, Knighton DR, Percoraro RE, Rodeheaver G, Robson MC (1994) Definitions and guidelines for assessment of wounds and evaluation of healing. Wound Repair Regen 2:165–170

Leveque JL, Corcuff P, de Rigal J, Agache P (1984) In vivo studies of the evolution of physical properties of the human skin with age. Int J Dermatol 23:322–329

Li J, Zhang YP, Kirsner RS (2003) Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. Microsc Res Tech 60:107–114

Litjens SH, de Pereda JM, Sonnenberg A (2006) Current insights into the formation and breakdown of hemidesmosomes. Trends Cell Biol 16:376–383

Lobmann R, Ambrosch A, Schultz G, Waldmann K, Schiweck S, Lehnert H (2002) Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. Diabetologia 45:1011–1016

Loeser AA (1937) The resorption and action of follicular hormone rubbed into the skin. J Obstet Gynaecol Br Emp 44:710

Loots MA, Lamme EN, Zeegelaar J, Mekkes JR, Bos JD, Middelkoop E (1998) Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds. J Invest Dermatol 111:850–857

Luetolf O, Bull RH, Bates DO, Mortimer PS (1993) Capillary underperfusion in chronic venous insufficiency: a cause for leg ulceration? Br J Dermatol 128:249–254

Magnusson U, Einarsson S (1990) Effects of exogenous oestradiol on the number and functional capacity of circulating mononuclear and polymorphonuclear leukocytes in the sow. Vet Immunol Immunopathol 25:235–247

Margolis DJ, Knauss J, Bilker W (2002) Hormone replacement therapy and prevention of pressure ulcers and venous leg ulcers. Lancet 359:675–677

Marriott I, Huet-Hudson YM (2006) Sexual dimorphism in innate immune responses to infectious organisms. Immunol Res 34:177–192

Martin DE, Reece MC, Maher JE, Reese SC (1988) Tissue debris at the injury site is coated by plasma fibronectin and subsequently removed by tissue macrophages. Arch Dermatol 124:226–229

Mills SJ, Ashworth JJ, Gilliver SC, Hardman MJ, Ashcroft GS (2005) The sex steroid precursor DHEA accelerates cutaneous wound healing via the estrogen receptors. J Invest Dermatol 125:1053–1062

Moses MA, Marikovsky M, Harper JW, Vogt P, Eriksson E, Klagsbrun M, Langer R (1996) Temporal study of the activity of matrix metalloproteinases and their endogenous inhibitors during wound healing. J Cell Biochem 60:379–386

Narita S, Goldblum RM, Watson CS, Brooks EG, Estes DM, Curran EM, Midoro-Horiuti T (2007) Environmental estrogens induce mast cell degranulation and enhance IgE-mediated release of allergic mediators. Environ Health Perspect 115:48–52

Nikolaevich KN, Ivanovich SJ, Victorovich SS (1991) Major reproduction hormones as regulators of cell-to-cell interactions in humoral immune responses. Brain Behav Immun 5:149–161

Oberholzer A, Keel M, Zellweger R, Steckholzer U, Trentz O, Ertel W (2000) Incidence of septic complications and multiple organ failure in severely injured patients is sex specific. J Trauma 48:932–937

Ossowski L, Aguirre-Ghiso JA (2000) Urokinase receptor and integrin partnership: coordination of signaling for cell adhesion, migration and growth. Curr Opin Cell Biol 12:613–620

Pastar I, Stojadinovic O, Tomic-Canic M (2008) Role of keratinocytes in healing of chronic wounds. Surg Technol Int 17:105–112

Pergola C, Dodt G, Rossi A, Neunhoeffer E, Lawrenz B, Northoff H, Samuelsson B, Rådmark O, Sautebin L, Werz O (2008) ERK-mediated regulation of leukotriene biosynthesis by

androgens: a molecular basis for gender differences in inflammation and asthma. Proc Natl Acad Sci U S A 105:19881–19886

Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, Bonazzi V, McEwen JE, Wetterstrand KA, Deal C, Baker CC, Di Francesco V, Howcroft TK, Karp RW, Lunsford RD, Wellington CR, Belachew T, Wright M, Giblin C, David H, Mills M, Salomon R, Mullins C, Akolkar B, Begg L, Davis C, Grandison L, Humble M, Khalsa J, Little AR (2009) The NIH human microbiome project. Genome Res 19:2317–2323

Phillips C, White RP Jr, Shugars DA, Zhou X (2003) Risk factors associated with prolonged recovery and delayed healing after third molar surgery. J Oral Maxillofac Surg 61:1436–1448

Punnonen R (1971) On the effect of castration and peroral estrogen therapy on the skin. Acta Obstet Gynecol Scand Suppl 9:32

Punnonen R, Vaajalahti P, Teisala K (1987) Local oestriol treatment improves the structure of elastic fibers in the skin of postmenopausal women. Ann Chir Gynaecol Suppl 202:39–41

Raja SK, Garcia MS, Isseroff RR (2007) Wound re-epithelialization: modulating keratinocyte migration in wound healing. Front Biosci 12:2849–2868

Robson MC (1997) Wound infection. A failure of wound healing caused by an imbalance of bacteria. Surg Clin North Am 77:637–650

Rømer J, Lund LR, Eriksen J, Pyke C, Kristensen P, Danø K (1994) The receptor for urokinase-type plasminogen activator is expressed by keratinocytes at the leading edge during re-epithelialization of mouse skin wounds. J Invest Dermatol 102:519–522

Rosenthal M, Goldberg D, Aiello A, Larson E, Foxman B (2011) Skin microbiota: microbial community structure and its potential association with health and disease. Infect Genet Evol 11:839–848

Roth RR, James WD (1988) Microbial ecology of the skin. Annu Rev Microbiol 42:441–464

Rundhaug JE (2005) Matrix metalloproteinases and angiogenesis. J Cell Mol Med 9:267–285

Salo T, Mäkelä M, Kylmäniemi M, Autio-Harmainen H, Larjava H (1994) Expression of matrix metalloproteinase-2 and -9 during early human wound healing. Lab Invest 70:176–182

Sauerbronn AV, Fonseca AM, Bagnoli VR, Saldiva PH, Pinotti JA (2000) The effects of systemic hormonal replacement therapy on the skin of postmenopausal women. Int J Gynaecol Obstet 68:35–41

Schober A, Weber C (2005) Mechanisms of monocyte recruitment in vascular repair after injury. Antioxid Redox Signal 7(9–10):1249–1257

Schramm JC, Dinh T, Veves A (2006) Microvascular changes in the diabetic foot. Int J Low Extrem Wounds 5:149–159

Schroder J, Kahlke V, Staubach KH, Zabel P, Stuber F (1998) Gender differences in sepsis human. Arch Surg 133:1200–1205

Seidenari S, Pagnoni A, Di Nardo A, Giannetti A (1994) Echographic evaluation with image analysis of normal skin: variations according to age and sex. Skin Pharmacol 7:201–209

Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, Gottrup F, Gurtner GC, Longaker MT (2009) Human skin wounds: a major and snowballing threat to public health and the economy. Wound Repair Regen 17:763–771

Shah MG, Maibach HI (2001) Estrogen and skin. An overview. Am J Clin Dermatol 2:143–150

Shuster S, Black MM, McVitie E (1975) The influence of age and sex on skin thickness, skin collagen and density. Br J Dermatol 93:639–643

Sjostrom L, Smith U, Krotkiewski M, Bjorntorp P (1972) Cellularity in different regions of adipose tissue in young men and women. Metabolism 21:1143–1153

Son ED, Lee JY, Lee S, Kim MS, Lee BG, Chang IS, Chung JH (2005) Topical application of 17beta-estradiol increases extracellular matrix protein synthesis by stimulating tgf-Beta signaling in aged human skin in vivo. J Invest Dermatol 124:1149–1161

Sumino H, Ichikawa S, Abe M, Endo Y, Ishikawa O, Kurabayashi M (2004) Effects of aging, menopause, and hormone replacement therapy on forearm skin elasticity in women. J Am Geriatr Soc 52:945–949

Taylor RJ, Taylor AD, Smyth JV (2002) Using an artificial network to predict healing times and risk factors for venous leg ulcers. J Wound Care 11:101–105

Thomason HA, Cooper NH, Ansell DM, Chiu M, Merrit AJ, Hardman MJ, Garrod DR (2012) Direct evidence that PKCα positively regulates wound re-epithelialization: correlation with changes in desmosomal adhesiveness. J Pathol 227:346–356

Trengove NJ, Stacey MC, McGechie DF, Mata S (1996) Qualitative bacteriology and leg ulcer healing. J Wound Care 5:277–280

Varila E, Rantala I, Oikarinen A, Risteli J, Reunala T, Oksanen H, Punnonen R (1995) The effect of topical oestradiol on skin collagen of postmenopausal women. Br J Obstet Gynaecol 102:985–989

Vaughan MB, Howard EW, Tomasek JJ (2000) Transforming growth factor-beta1 promotes the morphological and functional differentiation of the myofibroblast. Exp Cell Res 257:180–189

Werner S, Peters KG, Longaker MT, Fuller-Pace F, Banda MJ, Williams LT (1992) Large induction of keratinocyte growth factor expression in the dermis during wound healing. Proc Natl Acad Sci U S A 89:6896–6900

Wichmann MW, Zellweger R, DeMaso CM, Ayala A, Chaudry IH (1996) Mechanism of immunosuppression in males following trauma–hemorrhage. Critical role of testosterone. Arch Surg 131:1186–1191

Wysocki AB (1999) Skin anatomy, physiology, and pathophysiology. Nurs Clin North Am 34:777–797

Wysocki AB, Staiano-Cioco GF (1993) Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9. J Invest Dermatol 101:64–68

Yager DR, Zhang LY, Liang HX, Diegelmann RF, Cohen IK (1996) Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids. J Invest Dermatol 107:743–748

Ya-Xian Z, Suetake T, Tagami H (1999) Number of cell layers of the stratum corneum in normal skin – relationship to the anatomical location on the body, age, sex, and physical parameters. Arch Dermatol Res 291:555–559

Zeeuwen PLJM, Boekhorst J, van den Bogaard EJ, de Koning HD, van de Kerkhof PMC, Saulnier DM, van Swam II, van Hijum SAFT, Kleerebezem M, Schalkwijk J, Timmerman HM (2012) Microbiome dynamics of human epidermis following skin barrier disruption. Genome Biol 13: R101

Chapter 3
Immunology of Pregnancy and Systemic Consequences

Fiona M. Menzies and Fiona L. Henriquez

Abstract The uterus is a site rich in immune cells and is subject to regulation by the sex hormones progesterone and estrogens. Immune modulation within the uterus is initiated during coitus and continues through to the delivery of the baby and for some time postpartum. Several mechanisms to protect the semiallogeneic fetus from maternal immune attack exist, with the carefully regulated recruitment and function of immune cells, as well as the mediators they produce, now recognized as crucial to the success of pregnancy. In the postpartum period, immune cells are also vital to aid the repair and remodeling process, as well as ensure defense against pathogens. Dysregulation of these maternal immune mechanisms can lead to the development of conditions such as preeclampsia, preterm birth, and spontaneous abortion. This chapter will evaluate the immune environment within the uterus and the systemic consequences from coitus to postpartum uterine involution. The effect of pregnancy-associated immune modulation on the symptoms of the autoimmune diseases such as arthritis and systemic lupus erythematosus (SLE) pregnancy-associated infections is also reviewed.

3.1 Introduction

Sir Peter Brian Medawar is widely regarded as the founder of the reproductive immunology field. In 1953, this pioneer of transplantation biology asked the question of how the fetus is tolerated by the mother. In doing so, he was the first to recognize that the fetus is essentially an antigenic foreign body within the womb (Billington 2003). Although he did little research in this area, the complexity of the maternal-fetal interface has since been the subject of intense investigation.

Within the female reproductive tract, there is an abundance of immune cells as well as chemokines, small proteins involved in the trafficking of immune cells.

F.M. Menzies (✉) • F.L. Henriquez
Institute of Biomedical and Environmental Health Research, University of the West of Scotland, Paisley, Scotland
e-mail: Fiona.Menzies@uws.ac.uk

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_3

From coitus to postpartum uterine involution, immune cells are present and function to provide an environment suitable for the establishment of a successful pregnancy and ensure the return of the uterus to a prepregnancy state. During pregnancy, the process of placentation also requires immune adaptation by the mother. The uterus is therefore a unique environment that maintains a delicate balance between protecting the host from infection and establishing tolerance of the semiallogenic fetus. In addition, we must also consider immune involvement in pregnancy complications, autoimmune diseases, and how the hormonal and immunological changes that occur during pregnancy can influence the way the body responds to infection.

For the first 8 weeks of pregnancy, progesterone, which is often described as the pregnancy hormone, is produced by corpus luteum within the ovary. After this time, progesterone production is taken over by the placenta and it continues to rise dramatically until birth. Estrogens are produced primarily by developing follicles and the corpus luteum. Estradiol is the main estrogen in women of fertile age and estriol is produced by the placenta during pregnancy, whereas estrone is produced in women of menopausal age (Kuijper et al. 2013). Levels of estrogens in maternal serum and urine increase dramatically during pregnancy, falling dramatically after delivery of the fetus (Kuijper et al. 2013). The elevated levels of progesterone and estrogen during pregnancy have a profound influence on the immune environment within the uterus by modulating immune cell function (Druckmann and Druckmann 2005; Menzies and Henriquez 2009; Oertelt-Prigione 2012; Pennell et al. 2012). While these hormonal changes occur to support pregnancy, this can have consequences for the pathogenesis of autoimmune diseases or infections.

3.2 The Female Immune Response to Coitus

The release of seminal plasma into the vagina after coitus stimulates an inflammatory response within the female reproductive tract. While seminal plasma has traditionally been considered as a transport medium for spermatozoa, it is now appreciated that it has important roles in preparing the uterus, by inducing changes in gene expression and cellular composition, for pregnancy (Robertson 2005). The presence of immunomodulatory molecules within seminal plasma has huge implications for the success of pregnancy, whether it is via natural conception or assisted reproduction. Indeed, the importance of the inflammatory response to mating is demonstrated by studies showing that in vitro fertilization (IVF) is more successful in women who are exposed to semen at the time of embryo transfer (Tremellen et al. 2000; Bellinge et al. 1986).

Seminal plasma contains an array of biologically and immunologically active compounds including hormones, such as estrogen and progesterone, as well as an array of cytokines (Maegawa et al. 2002; Robertson 2005; Hampl et al. 2013). Of particular interest is the cytokine TGF-β, which is abundant within seminal plasma of both mice (Tremellen et al. 1998) and humans (Loras et al. 1999). Studies in

humans show that all three isoforms of TGF-β (TGF-β1, TGF-β2, and TGF-β3) are present within seminal plasma and can induce proinflammatory cytokine production by cervical cells (Sharkey et al. 2012a).

In humans, deposition of seminal fluid results in the influx of macrophages, dendritic cells (DCs), and T cells into the epithelial and stromal compartments of the ectocervix (portion of cervix extending into the vagina), accompanied by an increase in gene expression for the cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-1α and IL-6, and the chemokine CXCL8 (Sharkey et al. 2012b). Macrophages and DCs remain within the stromal region of the cervix, while increases in CD3+ T cells are found in both the epithelium and stroma (Sharkey et al. 2012b). Furthermore, a greater proportion of T cells are CD8+ than CD4+ (Sharkey et al. 2012b).

The first encounter between the maternal immune system and paternal antigens occurs when seminal plasma is introduced during coitus. As well as preparing the uterine epithelium for pregnancy, it has been found that seminal plasma also functions to modulate the uterine immune environment to prevent fetal rejection by reacting to antigens within seminal plasma and priming the maternal immune system (Fig. 3.1). Studies using a TCR-transgenic mouse model, with ovalbumin (OVA) as a model paternal antigen, show that seminal plasma drives the activation and expansion of OVA-specific CD4+ and CD8+ T cells. Maternal antigen-presenting cells uptake paternal antigens primarily by phagocytosis, before cross-presentation to CD8+ T cells in a TAP-dependent manner (Moldenhauer et al. 2009). After mating, there is a substantial increase in the cellularity of the lymph nodes draining the uterus in mice (Johansson et al. 2004), mainly the para-aortic lymph nodes (Johansson et al. 2004), the main site of cross-presentation of paternal antigen (Moldenhauer et al. 2009). Recent studies in mice have demonstrated that Th17 cells also respond to the presence of sperm in the female reproductive tract, with their action modulated and controlled by estradiol (Lasarte

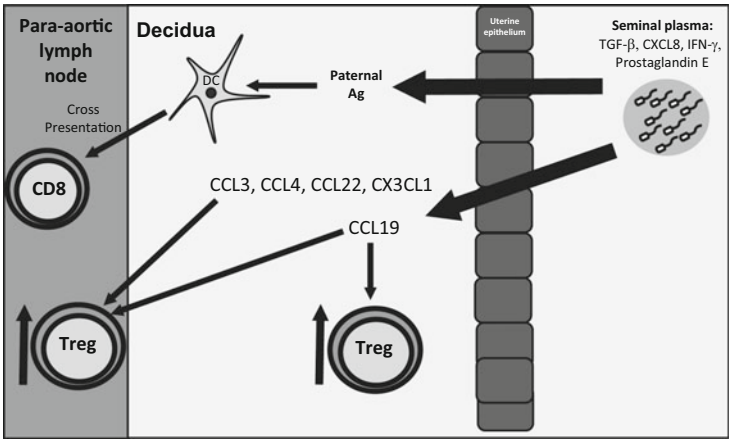

Fig. 3.1 Initiation of protective responses against the fetus

et al. 2013). It has been suggested that the initial presentation of paternal alloantigens to maternal T cells after ejaculation is crucial for tolerance of the conceptus, which displays the same paternal antigens, during implantation a few days later (Sharkey et al. 2012b).

Another way in which seminal plasma mediates fetal tolerance is through its ability to induce expansion of T regulatory (Treg) cells (Aluvihare et al. 2004; Robertson et al. 2009a). Mouse studies have demonstrated that Treg cells accumulate within the nonpregnant uterus as the female approaches estrus, the "fertile" stage of the murine cycle, and this is accompanied with expression of T-cell-specific chemokines CCL3, CCL4, CCL22, and CX3CL1 (Kallikourdis and Betz 2007). Through the production of CCL19 by glandular and luminal epithelial cells of the uterus in response to both semen and spermatozoa, Treg cells are recruited (Guerin et al. 2011). The importance of Treg cells in maintaining an environment suitable for pregnancy will be discussed in later sections (Fig. 3.1).

3.3 The Peri-Implantation Period

After successful fertilization, several rounds of cleavage (the first step in embryogenesis) occur as the young embryo (still only a small collection of cells) moves through the fallopian tube toward the uterine cavity. Implantation of the blastocyst into the uterine wall presents a crucial step in the reproductive process and requires coordination between the blastocyst and uterus under optimal conditions. Implantation can only occur within a set time period, called the implantation window, and in humans, this is approximately 8–10 days post-ovulation (Wilcox et al. 1999). In humans, the chance of conception per cycle is approximately 30 % (Zinaman et al. 1996), with around 75 % of early losses attributed to implantation failure (Wilcox et al. 1988). Having a clear understanding of the molecular, biochemical, and immunological mechanisms underlying implantation has obvious implications for the ability to improve assisted conception practices. However, practical and ethical restrictions in conducting human studies have meant that much of our knowledge of implantation stems from the use of murine models.

The uterine wall undergoes a number of morphological and biochemical changes in order to be receptive to the blastocyst. Implantation into the receptive uterine wall requires apposition, attachment, and penetration of the blastocyst through the uterine epithelium and requires adequate changes to the uterine endometrium (Norwitz et al. 2001; Cha et al. 2012). The formation of the decidua is necessary for successful implantation and is highly dependent upon sufficient levels of progesterone (Cha et al. 2012). Estrogen also plays a key role in this process, with murine studies showing that it is a key factor in determining the duration of the implantation window (Ma et al. 2003). Within the implantation window in humans, endometrial stromal cells undergo decidualisation to change from fibroblast-like precursor cells to larger, more rounded decidual stromal cells (DSCs) (Dunn et al. 2003). This is a critical point for the fate of the pregnancy.

Either decidualisation continues in preparation for blastocyst implantation or it breaks down to begin menstruation (King 2000). Furthermore, DSCs play an important role in controlling the passage of leukocytes to the implantation area (Dunn et al. 2003). Upon breach of the uterine epithelium by the blastocyst, a brief inflammatory-type reaction occurs in response to invasion of the trophoblasts. Cells such as macrophages are involved in the clearance of cellular debris as maternal uterine cells apoptose to allow invasion of trophoblast cells (Abrahams et al. 2004).

3.3.1 Cytokines and Chemokines During the Implantation Window

A number of key cytokines, growth factors, and chemokines (Fig. 3.2a) facilitate the process of implantation, and these have been extensively reviewed within the literature (Handwerger 1994; Simon et al. 1995, 1997; Dimitriadis et al. 2005; Robb et al. 2002). Inflammation is a necessary component of implantation (Mor et al. 2011), while excessive levels of inflammation within the decidua can lead to implantation failure or miscarriage. Of particular note are the roles played by leukemia inhibitory factor (LIF), IL-6 and IL-15, although more recently, work has been done to look at the role of IL-33 and its receptor ST2 during implantation, in which human endometrial stromal cells secrete IL-33 upon decidualisation. However, failure to control this production leads to pregnancy failure (Salker et al. 2012).

To date, one of the most characterized factors involved in implantation is LIF, and its role during pregnancy has been extensively reviewed in the literature (Aghajanova 2004; Kimber 2005; Suman et al. 2013). LIF is pleiotropic in nature, exhibiting multiple biological functions (Haines et al. 2000). Studies using LIF-deficient mice have shown that this highly glycosylated 40–50 kDa glycoprotein is essential for successful implantation (Stewart et al. 1992) through binding the LIF-receptor (LIFR) and inducing STAT3 activation (Suman et al. 2013). LIF-deficient mice exhibit no embryonic defects; however, implantation cannot occur without the administration of exogenous LIF (Stewart et al. 1992). Furthermore, studies have demonstrated that women diagnosed with recurrent implantation failure have significantly less LIF in their endometrial glandular epithelium than normal pregnant women (Mariee et al. 2012). During the implantation window, when LIF concentrations from the endometrium are at their peak, the blastocyst expresses the LIFR (Charnock-Jones et al. 1994). It is also thought that LIF induces both autocrine and paracrine signaling pathways in the endometrium to facilitate implantation (Cullinan et al. 1996; Dominguez et al. 2002). Even after implantation of the blastocyst LIF/STAT3 signaling is important in increasing the ability of trophoblast cells to invade (Poehlmann et al. 2005), which is crucial for successful placentation in the early stages of pregnancy.

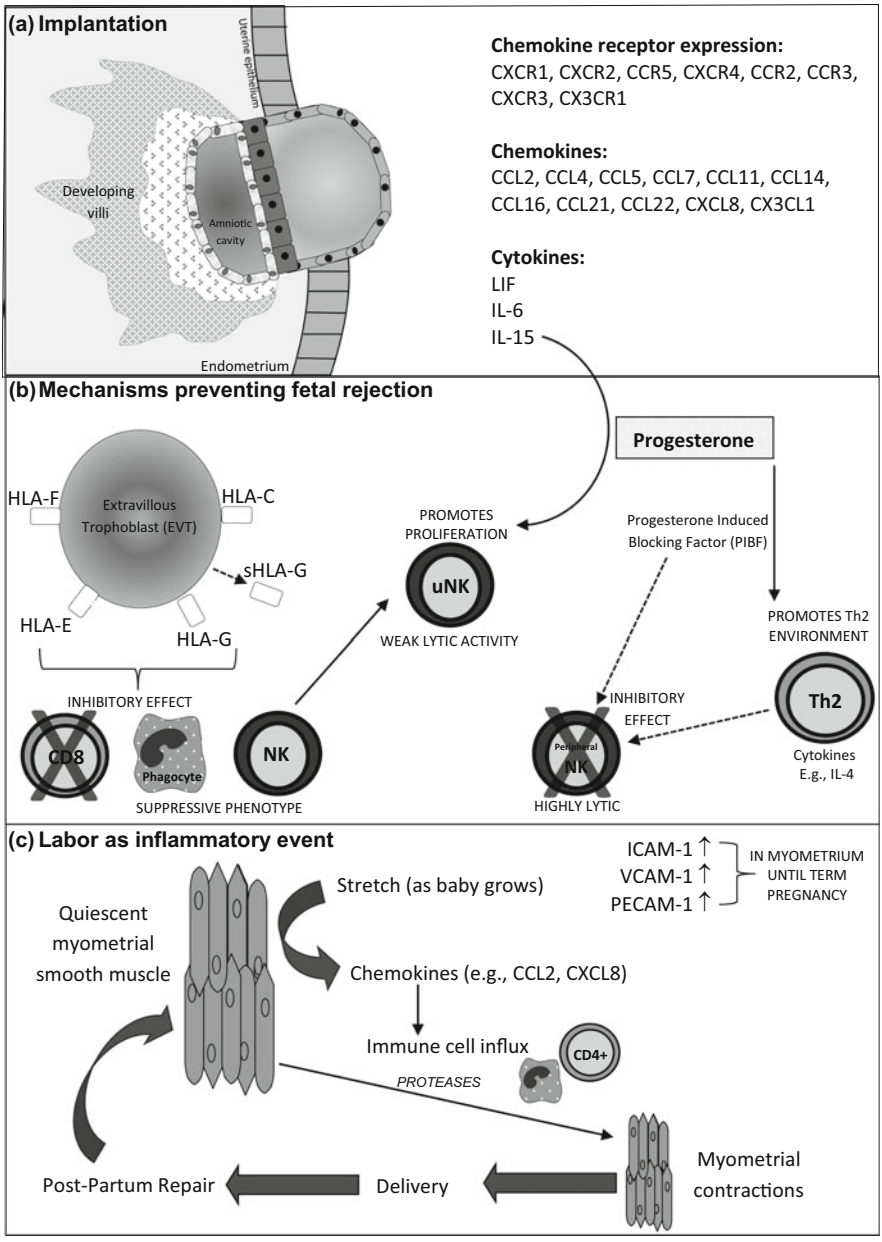

Fig. 3.2 Summary of immune involvement during pregnancy. (**a**) Chemokine receptor expression by the endometrium at the time of implantation, as the developing placental villi extend into the uterine wall, and the main chemokines and cytokines produced by cells of the endometrial stroma. (**b**) Main mechanisms involved in preventing rejection of the fetus as pregnancy progresses. Altered HLA expression by EVT cells promotes uNK cell phenotype and inhibits action of cytotoxic CD8+ T cells and phagocytes. Progesterone promotes a Th2 environment, and this along with the action of PIBF prevents the action of peripheral NK cells, and promotes the protective uNK cell phenotype. (**c**) Labor as an inflammatory event. As pregnancy progresses,

LIF is a member of the IL-6 family of cytokines. During the menstrual cycle, endometrial expression of IL-6 peaks during the implantation window, with glandular and surface epithelial cells showing greatest expression (Tabibzadeh et al. 1995). Studies in mice have shown that IL-6 deficiency reduces fertility, as well as significantly decrease the number of viable implantation sites (Robertson et al. 2000). It has been suggested, however, that IL-6 may have a partially redundant role during the implantation window (Dimitriadis et al. 2005).

IL-15 is a 14–15 kDa cytokine that is a member of the IL-2 family of cytokines. IL-15 has the ability to influence both innate and adaptive arms of the immune response, through its ability to stimulate natural killer (NK) cells, T cells, and NKT cells. IL-15 transcripts are strongly expressed during the secretory, or luteal, phase of the cycle in humans a time in which a large number of NK cells are present within the uterus (Kitaya et al. 2000). As we will discuss later in some detail, the regulation of NK cells and their phenotype within the uterus is critical for the success of pregnancy. Indeed, a recent study found that significantly higher levels of IL-15, which correlates with uterine NK (uNK) cell numbers, are found in the endometrium of women with recurrent implantation failure compared with normal women (Mariee et al. 2012).

The endometrium of nonpregnant humans also expresses several chemokine receptors, including CXCR1, CXCR2, CCR5, CXCR4, CCR2, CCR3, CXCR3, and CX3CR1 (Zhang et al. 2000; Dominguez et al. 2003a; Mulayim et al. 2003; Hannan et al. 2004; Hirota et al. 2006; Laird et al. 2011), and produces a number of chemokines (e.g., CCL2, CCL4, CCL5, CCL7, CCL11, CCL14, CCL16, CCL21, CCL22, CXCL8, CX3CL1), with high levels generally observed during the implantation window (Caballero-Campo et al. 2002; Hannan et al. 2004; Jones et al. 2004). Many of these chemokines are involved in the trafficking of immune cells, including Treg cells, macrophages, and uNK cells to the decidua. It has been suggested that chemokines and their receptors may also play other significant roles during the establishment of pregnancy, with receptors found on cells of the blastocyst and extravillous trophoblasts (EVT) (Dominguez et al. 2003a, b; Dimitriadis et al. 2005; Hannan and Salamonsen 2007).

3.3.2 Immune Cells Involved in Implantation

It has been estimated that in early pregnancy, 30–40 % of stromal cells within the decidua are leukocytes (Bulmer et al. 1991). The profile of immune cell populations within the nonpregnant endometrium and the distribution of cells between the

Fig. 3.2 (continued) the uterine wall stretches, leading to the upregulation of leukocyte adhesion molecules, and production of chemokines, leading to an influx of inflammatory immune cells. It is suggested that this contributes to the promotion of contractions; however, this could also aid postpartum repair and remodeling of the uterus

stratum basalis and the stratum functionalis layer of the endometrium are subject to change throughout the menstrual cycle (King 2000). In addition, the number and purpose of immune cells in these regions adapt during the implantation window and throughout pregnancy to support the developing placenta and fetus.

In the nonpregnant uterus, T and B cells are found in lymphoid aggregates, which develop during the proliferative phase of the menstrual cycle, by the trafficking of cells to the stratum basalis region of the endometrium (Yeaman et al. 1997, 2001). These aggregates consist of a core of B cells, surrounded by T cells, most of which are CD8+ in origin. These cells actively traffic to the endometrium, rather than having been derived from the division of preexisting cells within the uterus (Yeaman et al. 2001). Data on the purpose of these cells, their role during the implantation period, and on their cytotoxic abilities at this early gestational period is limited. Many studies to date have focussed on the characterisation and role of decidual CD8+ T cells during the latter stages of pregnancy. Indeed, only 5–20 % of decidual leukocytes in early pregnancy are T cells, but this figure rises to 40–80 % by term pregnancy (Tilburgs and Strominger 2013), suggesting these cells play a more prominent role as pregnancy progresses. Indeed, studies have demonstrated that by the end of the first trimester in humans (approximately 7–12 weeks), CD8+ T cells are important in the placentation process by regulating the invasion of EVT (Scaife et al. 2006), and by term pregnancy (approximately 37–42 weeks) decidual CD8+ T cells have a very different phenotype to their peripheral counterparts, expressing less perforin and granzyme B, thereby reducing their ability to adversely affect the semiallogeneic fetus (Tilburgs et al. 2010).

Treg cells are a particularly interesting population of cells within the human endometrium during the reproductive cycle and throughout pregnancy and have been the subject of much study and review in both humans and mice over the past 10 years (Aluvihare et al. 2004; Guerin et al. 2009; Leber et al. 2010; Zenclussen et al. 2010; Teles et al. 2013). As already discussed, Treg cells accumulate within the endometrium during the preimplantation period (Kallikourdis and Betz 2007). Normal pregnant mice expand their numbers of Treg cells immediately after conception in a number of organs including the lymph nodes, spleen, blood, and thymus, although mRNA for the Treg marker Foxp3 does not increase in uterine tissues until day 5 of pregnancy (Thuere et al. 2007). This suggests that these cells expand in the periphery and migrate to the uterus (Aluvihare et al. 2004; Thuere et al. 2007) where they act to control maternal anti-fetal responses. The protective role of Treg cells at the implantation site has recently been demonstrated in a murine model of pregnancy, where Treg cells become activated against self-antigens within a few days after embryo implantation in both allogeneic and syngeneic matings and act to create an early tolerant immune environment (Chen et al. 2013). Fetal-antigen-specific Treg cells are not present at embryo implantation; however, their number increases as pregnancy progresses (Rowe et al. 2012).

Macrophages play an important role within the endometrium as it cycles with changes in reproductive hormones. Progesterone and estrogen not only modulate the inflammatory activities of macrophages (Jones et al. 2008; Menzies et al. 2011a; Liu and Wang 2013), but have an influential role on the distribution of these cells

within the nonpregnant uterus (De and Wood 1990). Macrophage numbers rapidly increase around the time of implantation, firstly to contribute to the clearance of cellular debris to aid in embryo implantation (Abrahams et al. 2004) but also to degrade any semen or pathogens present within the uterus as a result of mating (De et al. 1991). More recently, the necessity for macrophages during the process of implantation has been investigated. Depletion of macrophages in mice during the preimplantation period prevents successful implantation and therefore pregnancy, by disrupting the ability of the corpus luteum to produce sufficient quantities of progesterone (Care et al. 2013). The addition of exogenous progesterone to these mice rescued their ability to have a successful pregnancy. This study is particularly interesting as few reproductive studies consider the effect of immune cell function on hormone production.

Uterine NK cells are the subject of much interest in studies of reproductive immunology, including pregnancy and fertility studies, and as will be discussed later, this is partly due to the differences in their phenotype when compared with peripheral NK cells. The phenotype and regulation of NK cells within the pregnant uterine environment are crucial for pregnancy success. However, little is known about the role of uNK cells in the facilitation of embryo implantation in humans, despite the fact that they constitute 50–70 % of immune cells within first trimester decidua (Bulmer et al. 1991, 2010; Manaster and Mandelboim 2010) and they are found in close proximity to the implantation site (Dosiou and Giudice 2005; Oh and Croy 2008). Estrogen and progesterone act to recruit uNK cell precursors to the uterus (Kuang et al. 2010). However, uNK cells are known to play vital roles in regulating the invasion of trophoblasts as well as maternal immune responses to the fetus.

3.4 Mechanisms Involved in Preventing Fetal Rejection

Upon implantation of the blastocyst into the receptive decidual lining, the process of placentation begins. The invasion of placental EVT into the uterine wall has to be carefully regulated in order to prevent attack and destruction by maternal leukocytes. Dysregulated EVT invasion is associated with abnormal placentation, leading to defects in placenta function, compromising both the growth and survival of the offspring (Rossant and Cross 2001; Smith et al. 2004; Bukowski 2011).

One of the most important factors in protection of EVT cells, the main fetally derived cells in contact with maternal tissues, is the altered human leukocyte antigen (HLA) expression by these cells. EVT cells do not display the MHC class I molecules HLA-A and HLA-B, or the class II HLA-D molecules, but do display the class I molecules HLA-C, HLA-E, HLA-F, and HLA-G. HLA-G is the most studied of these HLA molecules in the placenta. Seven isoforms have been identified, with HLA-G1, HLA-G2, HLA-G3, and HLA-G4 being membrane bound and HLA-G5, HLA-G6, and HLA-G7 being soluble (Hviid 2006; Hunt and Langat 2009). Table 3.1 summarizes key studies examining the expression of these

Table 3.1 Trophoblast HLA expression

HLA molecule	Expression pattern	References
HLA-C	Surface EVT expression	King et al. (2000b)
	EVT expression	Apps et al. (2009)
HLA-E	Expressed by trophoblasts, ligand for CD94/NKG2 NK cell receptor	King et al. (2000a)
	Found on all placental cells co-expressing HLA-G	Ishitani et al. (2003)
	EVT expression	Apps et al. (2009)
HLA-F	Surface EVT expression	Ishitani et al. (2003)
	EVT expression, mainly in cytoplasm during first trimester, and then surface during second and third trimester	Shobu et al. (2006)
HLA-G	Surface EVT expression	King et al. (2000b)
	Membrane-bound HLA-G expressed on EVT and sHLA-G on all placental trophoblasts	Ishitani et al. (2003)
	EVT expression	Apps et al. (2009)
	Placental Hofbauer cells	Yang et al. (1996)
	Endothelial cells of chorionic villi	Blaschitz et al. (1997)
	Amniotic cells	Hammer et al. (1997)

EVT Extravillous trophoblasts, *HLA* human leukocyte antigen, *NK* natural killer, *sHLA* soluble human leukocyte antigen

molecules at the maternal-fetal interface. In general terms, the expression of these molecules by placental cells promotes tolerance of the fetus. These trophoblast HLA molecules act as inhibitory ligands (Fig. 3.2b), causing cytotoxic T cells to die or reduce CD8 expression, and phagocytes and NK cells to obtain a suppressive phenotype (King et al. 2000a; Hunt et al. 2005; Chazara et al. 2011).

NK cells are key cells in the early innate defense against various pathogens but are particularly useful in the response to viral infection. Within the blood, two main types of NK cells have been characterized based on expression of CD56 and are referred to as $CD56^{dim}$ or $CD56^{bright}$. $CD56^{dim}$ cells are the main peripheral NK cell population, comprising approximately 90 % of NK cells within the blood and are characterized by a high level of spontaneous lytic activity (Dosiou and Giudice 2005). By comparison, the $CD56^{bright}$ population have little lytic activity. uNK cells differ from both of these NK phenotypes but have more in common with this latter phenotype, including weak lytic activity (Ferry et al. 1990); however, uNK cells show no expression of the NK marker CD16 (Saito 2000). Binding of IgG to CD16 results in activation of antibody-dependent cell-mediated cytotoxicity (ADCC) by NK cells, and so the absence of CD16 on uNK cells demonstrates another tolerogenic feature of these cells. Many comparisons between blood $CD56^{dim}$, $CD56^{bright}$, and uNK cells have been reviewed within the literature (Moffett-King 2002; Dosiou and Giudice 2005; Vacca et al. 2013). Dysregulation between uNK cells and peripheral NK cells during pregnancy has been investigated as a mechanism to account for recurrent pregnancy loss (Tang et al. 2011).

A number of protective and supportive functions of uNK cells during pregnancy have been identified. Their close proximity to the implantation site, and site of EVT invasion, suggests that they are important in regulation of trophoblast invasion and the maternal immune response to this invasion (Moffett-King 2002). uNK are greatly influenced in their trafficking and function by sex steroid hormones (Fig. 3.2b) (Dosiou and Giudice 2005). Both estrogen and progesterone act to promote the homing of $CD56^{bright}$ cells to the uterus (Chantakru et al. 2003), and progesterone also promotes proliferation of uNK cells through production of IL-15 by endometrial stromal cells (Manaster et al. 2008; Vacca et al. 2011). Progesterone can also influence NK cell activity in an indirect manner. Progesterone supports the development of a helper T-cell type 2 (Th2)-type environment (Piccinni et al. 1995), and many of the Th2-type cytokines (e.g., IL-4) have been shown to limit the activity of peripheral NK cells (Loza et al. 2002). Many functions of progesterone, including promotion of a Th2 environment, are exerted through induction of the progesterone-induced blocking factor (PIBF) protein (Szekeres-Bartho and Wegmann 1996), which can also influence NK cell activity. Murine studies have demonstrated the ability of PIBF to inhibit the lytic activities of peripheral NK cells (Szekeres-Bartho et al. 1997).

Regulation of the cytokine environment and abundance of Treg cells within the uterus is another key mechanism of regulation of the uterine immune environment during pregnancy. Traditionally, it was considered that the placental and uterine environment should be skewed toward an abundance of Th2-type cytokines for the successful continuation of pregnancy (Lin et al. 1993; Wegmann et al. 1993). This was supported by data showing that women who suffer spontaneous recurrent abortions had an abundance of Th1-type cytokines and/or a deficiency in the production of LIF, IL-4, and IL-10 by decidual T cells (Piccinni et al. 1998; Ng et al. 2002). This paradigm has now been revisited to include Th17 cells, Treg cells, and their cytokines (Saito et al. 2010).

Experiments involving the culture of T cells in the presence of medium conditioned by placental trophoblasts demonstrated a skewing toward the production of Th2-associated transcription factors and cytokines and inhibition of those associated with Th1 an d Th17 (Liu et al. 2011). Progesterone can inhibit the differentiation of CD4+ T cells into Th17 cells (Lee et al. 2011) and downregulates the expression of the Th17-associated transcription factors RORγt (Maeda et al. 2013). The number of circulating Th17 cells does not change throughout pregnancy (Nakashima et al. 2010); however, an increased number of Th17 cells, and production of IL-17, within the blood and decidua during pregnancy has been associated with preterm labor and recurrent spontaneous abortion (Ito et al. 2010; Wang et al. 2010).

The balance between Th17 cells and Treg cells is critical for the outcome of pregnancy (Wang et al. 2010). During healthy pregnancy, the ratio of circulating Treg cells to Th17 cells is increased significantly compared to nonpregnant controls; however, in preeclamptic women, this skewing away from Th17 cells is not observed (Santner-Nanan et al. 2009). Treg cells within the decidua have been shown to increase with advancing gestation (Somerset et al. 2004; Tilburgs

et al. 2006) but again are lower in decidua from preeclamptic women (Quinn et al. 2011), suggesting a key role for these cells in regulating the maternal immune environment at the maternal-fetal interface. There is a preferential recruitment of fetus-specific Treg cells from the maternal blood to the decidua (Tilburgs et al. 2008). As Treg cells are considered as pregnancy protective, it is of no surprise that progesterone and estrogen, which are elevated during pregnancy, act to support the expansion of these cells (Polanczyk et al. 2004; Mao et al. 2010).

3.5 Parturition as an Inflammatory Event

The initiation of labor, or parturition, in humans is a complex, multifactorial process involving a mixture of endocrine and mechanical signals, which are intrinsically linked to induce the synchronistic contractions of the myometrium (Smith 2007). The underlying mechanisms of labor initiation in humans are not yet fully understood; however, many pathways have been considered (Smith 2007). For example, it is well known that activation of the hypothalamic-pituitary-adrenal axis, prostaglandin production, an increase in the responsiveness to oxytocin, and the functional withdrawal of progesterone are all key events prior to labor contractions. Indeed, the myometrium undergoes a series of phenotypic changes throughout each stage of pregnancy (Shynlova et al. 2012), culminating in a contractile phenotype, with an upregulation of contraction-associated proteins (e.g., connexin-43, oxytocin, and prostaglandin receptors).

In addition to these mechanisms, the infiltration of inflammatory cells and their mediators is now considered key in the initiation and propagation of normal labor at term (Fig. 3.2c). Uterine inflammation, as a result of infection, is present in 30–50 % of cases of preterm birth (Goldenberg et al. 2000) and was therefore considered a main driver of preterm labor. Preterm birth accounts for 5–10 % of all deliveries in developed countries (Challis et al. 2001) and therefore represents a significant obstetric problem (Goldenberg et al. 2000). In order to develop effective ways to prevent preterm birth, a better understanding of the mechanisms underlying normal labor is required.

As pregnancy proceeds, the uterus stretches to accommodate the growing fetus. This process drives the production of proinflammatory chemokines such as CCL2 and CXCL8, as shown by in vitro studies where mechanical stretch was applied to myometrial smooth muscle cells (Loudon et al. 2004; Shynlova et al. 2008; Hua et al. 2012). Transcriptional profiling of myometrial and cervical biopsies from women in spontaneous labor at term and not in labor (with no signs of clinical infection) revealed that 138 genes in myometrium and 164 genes in cervical tissue are upregulated, with 110 genes common to both tissues (Bollapragada et al. 2009). Interestingly, genes for the chemokines CXCL8, CXCL5, CXCL3, CCL20, CXCL1, CXCL2, CCL2, and CCL23 were among those genes commonly upregulated in both the myometrium and cervix with labor onset. In addition,

in vitro studies using a myometrial cell line have demonstrated that IL-1β upregulates chemokine gene expression (Chevillard et al. 2007).

Studies at both the genetic and cellular level suggest that normal labor is associated with an influx of inflammatory cells, concordant with this increase in chemokine, and proinflammatory cytokine production. Histological analysis of myometrial biopsies from nonpregnant and pregnant women at term, both not in labor and in spontaneous labor, demonstrates an increase in the number of neutrophils, macrophages, and T cells within the myometrium after labor onset (Thomson et al. 1999; Osman et al. 2003). Within the myometrium, the proinflammatory cytokines IL-1β, TNF-α, and IL-6 have been localized to these leukocytes, whereas in the cervix, these cytokines are found not only in leukocytes but also within the glandular and surface epithelium (Young et al. 2002). Gene expression levels of these cytokines are increased in the cervix and myometrium with labor onset and levels correlate with the increase in leukocyte numbers (Osman et al. 2003).

Cell adhesion molecules are required for the binding and extravasation of cells into tissues, and it has been shown that their expression is upregulated with labor onset (Thomson et al. 1999; Ledingham et al. 2001; Winkler et al. 2003). Intercellular adhesion molecule (ICAM)-1 mRNA expression increases approximately tenfold in both the cervix and myometrium upon labor initiation and is localized to the vascular endothelium and leukocytes (Ledingham et al. 2001), although immunohistochemical analysis for ICAM-1 does not show any change in tissue expression levels (Thomson et al. 1999). Vascular cell adhesion molecule (VCAM)-1 mRNA is upregulated within the cervix during pregnancy, although no further increases are observed with labor onset (Ledingham et al. 2001). As with ICAM-1, no changes are observed in VCAM-1 expression when analyzed histologically (Thomson et al. 1999). Platelet-endothelial cell adhesion molecule (PECAM)-1 mRNA expression increases ninefold in the myometrium with pregnancy; however, this molecule does not further increase with labor (Ledingham et al. 2001). ICAM-1, VCAM-1, and PECAM-1 are found on various cell types, including monocytes, lymphocytes, neutrophils, eosinophils, and endothelial cells. Collectively, these studies further demonstrate the inflammatory response associated with labor onset in humans.

However, more recently, a number of studies have questioned the necessity for inflammation in driving the initiation of contractions. Studies which utilize animal models of pregnancy and labor have provided evidence that while inflammation may promote the progression of labor, it may not be an absolute requirement for normal term labor. For example, studies examining the inflammatory role of mast cells and the chemokine receptor CCR2 during labor in mice have shown that the pregnant uterus is a robust environment with potentially many compensatory mechanisms to ensure successful delivery of fetuses (Menzies et al. 2011b, 2012).

3.6 Immune Involvement in Postpartum Uterine Involution

After birth, restoration of the uterus to the prepregnancy state is important for the success of future pregnancies. Interestingly, transabdominal and transvaginal sonographical examination of the human uterus has revealed that parity is irrelevant in the progress of uterine involution (Mulic-Lutvica et al. 2001) indicating the efficiency of the repair and remodeling mechanisms involved in uterine involution. Little is known about the molecular and immunological processes involved in postpartum uterine involution in humans due to the practical and ethical issues involved in collecting appropriate tissue samples; therefore, much of the work carried out to investigate this process has been done using mouse and rat models.

Studies in mice and rats show that repair of the uterine wall after birth requires a vast amount of extracellular matrix remodeling, proliferation, and apoptosis. In particular, the breakdown of collagen is required during uterine involution (Salamonsen 2003). Mice resistant to the action of collagenase enzymes exhibit defective postpartum uterine remodeling (Liu et al. 1995), which is usually completed by day 10 after delivery (Skurupiy et al. 2010) in normal mice. Matrix metalloproteinases (MMPs) are a group of endopeptidase enzymes that break down various components of the extracellular matrix. Several of these have been implicated in the process of postpartum involution of the uterus, including membrane type 1-MMP (MT1-MMP) (Manase et al. 2006), MMP-2 (Manase et al. 2006), MMP-7 (Woessner 1996), MMP-8 (Balbin et al. 1998), and MMP-9 (Manase et al. 2006).

While it has been shown that inflammatory cytokines including IL-1β and TNF-α can stimulate MMP production (Braundmeier and Nowak 2006), the role that immune cells such as macrophages and neutrophils play in the postpartum remodeling phase, whether it be contributing to MMP production or phagocytosis of apoptotic cells, has yet to be studied in detail. Indeed, these cells are known to be important in the remodeling of the cervix (Timmons et al. 2009), which is required prior to labor.

3.7 Consequences of Pregnancy for the Immune System

In order for pregnancy maintenance, the female body significantly changes its hormonal balance. As we have already discussed, sex hormones, namely, progesterone and estrogens, can have multiple influences on the cells of the immune system (Bouman et al. 2005; Menzies and Henriquez 2009). Therefore, pregnancy and the associated hormonal changes have wider implications of for the maternal immune system. Specifically, pregnancy affects maternal autoimmune (discussed below) diseases and the outcome of infectious diseases (see Chap. 13).

3.7.1 Immune Adaptation to Pregnancy

As we have seen, during pregnancy, the immune system within the uterus adapts significantly to accommodate implantation, fetal growth, parturition, and postpartum involution. However, adaptations to the systemic immune system also occur.

Throughout normal pregnancy in humans, there is an increase in the number of circulating monocytes and granulocytes (Matthiesen et al. 1995; Minagawa et al. 1999; Luppi et al. 2002a, b; Belo et al. 2005) and a decrease in the number of circulating lymphocytes (Minagawa et al. 1999). Monocytes are activated during pregnancy compared with nonpregnant controls, with increased production of IL-1β and IL-12 (Luppi et al. 2002b) and elevated serum levels of the inflammatory marker C-reactive protein (CRP) (Belo et al. 2005). By comparison, while circulating neutrophil numbers are elevated in pregnancy (Belo et al. 2005), there is a substantial body of evidence to suggest that these cells have reduced effector functions. Specifically there is a reduction in their chemotaxis, microbial killing, and production of reactive oxygen species (El-Maallem and Fletcher 1980; Crouch et al. 1995; Kindzelskii et al. 2004).

While the function of different T-cell subsets has been described in detail in this chapter, the role of B cells during pregnancy has yet to be fully addressed. B cells constitute 5–15 % of circulating lymphocytes and have been studied extensively for their role in antibody production. Much research has been done to examine both the protective functions of antibodies and harmful effects of autoantibodies during pregnancy. Recent advances in our understanding of different B-cell subsets (Bao and Cao 2014), namely, B1 and B2 B cells, have proved useful (Muzzio et al. 2013). It has been hypothesized that B1 cells are responsible for the production of autoantibodies, which are detrimental to pregnancy, whereas B2 cells are responsible for production of asymmetric antibodies, which have been shown to be beneficial for pregnancy (Muzzio et al. 2013). Asymmetric antibodies are IgG molecules with a modified Fab region, rendering them ineffective at opsonization (Labeta et al. 1986). Several studies have shown that these have a protective role in pregnancy (Gutierrez et al. 2005; Zenclussen et al. 2001) and that low serum levels of asymmetric antibodies can threaten the success of pregnancy (Barrientos et al. 2009).

In general, women exhibit much more vigorous humoral responses than men, with higher serum levels of IgG and total IgM (Eidinger and Garrett 1972; Giltay et al. 2000), with several studies showing the ability of β-estradiol to influence B-cell development (Medina and Kincade 1994). Many of the immune modifications that have been described can be attributed to the action of hormones, and the importance of understanding these influences is highlighted by consideration of the sex bias in the susceptibility to a number of diseases and infections.

3.7.2 Autoimmune Disease During Pregnancy

Rheumatoid arthritis (RA) involves the destruction of the cartilage surrounding the joints of the fingers, knees, and elbows, with women three times more likely to develop this condition than men. 75 % of women with RA who become pregnant exhibit improvement of their symptoms, with most improvement observed in the latter stages of pregnancy (Nelson and Ostensen 1997); however, within 3 months postpartum, this is reversed, and symptoms return (Adams Waldorf and Nelson 2008). The underlying mechanisms responsible for disease amelioration during pregnancy are unclear. However, studies suggest that the elevated levels of sex hormones during pregnancy are crucial to the improvement of RA symptoms. A recent study using a mouse model of human RA has shown that ovariectomized mice exhibit severe disease pathology and elevated serum TNF-α and IL-6, but addition of either estrogen or progesterone to these mice dramatically improves symptoms (Inoue et al. 2013). In addition, estrogen is also thought to be protective against the development of osteoarthritis (Linn et al. 2012).

Systemic lupus erythematosus (SLE) is a multiorgan autoimmune disease defined by the production of autoantibodies against nuclear antigens (Crispin et al. 2010). Women are nine times more likely to develop this condition than men. Although the underlying hormonal causes are not fully understood, 45 % of SLE patients exhibit autoantibodies to the estrogen receptor, ER-α, which interferes with T-cell homeostasis (Colasanti et al. 2012). By comparison with the arthritic conditions, SLE symptoms are not reduced by pregnancy. In some instances, symptoms are heightened during pregnancy, with SLE patients showing higher rates of fetal loss (Foocharoen et al. 2009; Yan Yuen et al. 2008; Sadowska 2005). Fifteen to sixty percent of SLE patients have reported exacerbations of the condition during pregnancy (Adams Waldorf and Nelson 2008).

Multiple sclerosis (MS) was first recognized in 1868 by Jean-Martin Charcot. This condition becomes manifested through immune attack of the myelin sheath surrounding nerves, with women three times as likely to be affected as men (Disanto and Ramagopalan 2013). This is partly genetic, with women more likely to carry the HLA DRB1 allele (Bove and Chitnis 2014). Despite this increased risk in developing the condition, men with MS develop a much more progressive disease with poor recovery after attacks. Similar to the other autoimmune diseases discussed, it has been found that pregnancy is associated with a significant reduction in the rate of MS relapses and increases during the 3 months postdelivery (Confavreux et al. 1998). Studies using the experimental allergic encephalomyelitis (EAE) model of MS have shown that progesterone, estriol, and estradiol may play an important role in the preventing relapses in sufferers of this condition (Kim et al. 1999; El-Etr et al. 2005).

Sex and pregnancy-associated hormones have been shown to have a major influence on both the incidence and progression of a number of autoimmune diseases, raising the question as to the necessity for sex-specific treatments. With global aging populations and increasing incidence of autoimmune disease, it may

be necessary to explore new treatment options tailored to either men or women. The overwhelming effect that pregnancy has on the symptoms of RA, for example, suggests that there are significant pregnancy-associated alterations that could be exploited as potential treatments.

3.8 Conclusions

In this chapter, the function of the immune system at each critical stage of pregnancy has been considered. Although much has still to be investigated, developing an understanding of the way in which the immune system adapts during pregnancy is vital, not just for diagnosis and treatment of pregnancy-related conditions but for providing researchers with an insight into the wider capabilities of the immune system, and how it can be manipulated. In doing so, we are arming ourselves with greater knowledge that can be used in our understanding of infections and autoimmune diseases.

Seminal plasma contains a number of immunomodulatory molecules including TGF-β, CXCL8, IFN-γ, and Prostaglandin E. The uterine epithelium responds by producing a number of chemokines, allowing recruitment of modulatory T regulatory cells to the site of deposition. Paternal antigens are sampled by DCs, which cross presents to CD8+ T cells within the lymph nodes draining the uterus. These mechanisms serve to prepare the maternal immune system for the development of a semiallogeneic fetus (Guerin et al. 2011; Robertson et al. 2009a, b; Kallikourdis and Betz 2007; Aluvihare et al. 2004).

References

Abrahams VM, Kim YM, Straszewski SL, Romero R, Mor G (2004) Macrophages and apoptotic cell clearance during pregnancy. Am J Reprod Immunol 51:275–282

Adams Waldorf KM, Nelson JL (2008) Autoimmune disease during pregnancy and the microchimerism legacy of pregnancy. Immunol Invest 37:631–644

Aghajanova L (2004) Leukemia inhibitory factor and human embryo implantation. Ann NY Acad Sci 1034:176–183

Aluvihare VR, Kallikourdis M, Betz AG (2004) Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol 5:266–271

Apps R, Murphy SP, Fernando R, Gardner L, Ahad T, Moffett A (2009) Human leucocyte antigen (HLA) expression of primary trophoblast cells and placental cell lines, determined using single antigen beads to characterize allotype specificities of anti-HLA antibodies. Immunology 127:26–39

Balbin M, Fueyo A, Knauper V, Pendas AM, Lopez JM, Jimenez MG, Murphy G, Lopez-Otin C (1998) Collagenase 2 (MMP-8) expression in murine tissue-remodeling processes. Analysis of its potential role in postpartum involution of the uterus. J Biol Chem 273:23959–23968

Bao Y, Cao X (2014) The immune potential and immunopathology of cytokine-producing B cell subsets: a comprehensive review. J Autoimmun 55:10–23

Barrientos G, Fuchs D, Schrocksnadel K, Ruecke M, Garcia MG, Klapp BF, Raghupathy R, Miranda S, Arck PC, Blois SM (2009) Low levels of serum asymmetric antibodies as a marker of threatened pregnancy. J Reprod Immunol 79:201–210

Bellinge BS, Copeland CM, Thomas TD, Mazzucchelli RE, O'neil G, Cohen MJ (1986) The influence of patient insemination on the implantation rate in an in vitro fertilization and embryo transfer program. Fertil Steril 46:252–256

Belo L, Santos-Silva A, Rocha S, Caslake M, Cooney J, Pereira-Leite L, Quintanilha A, Rebelo I (2005) Fluctuations in C-reactive protein concentration and neutrophil activation during normal human pregnancy. Eur J Obstet Gynecol Reprod Biol 123:46–51

Billington WD (2003) The immunological problem of pregnancy: 50 years with the hope of progress. A tribute to Peter Medawar. J Reprod Immunol 60:1–11

Blaschitz A, Lenfant F, Mallet V, Hartmann M, Bensussan A, Geraghty DE, Le Bouteiller P, Dohr G (1997) Endothelial cells in chorionic fetal vessels of first trimester placenta express HLA-G. Eur J Immunol 27:3380–3388

Bollapragada S, Youssef R, Jordan F, Greer I, Norman J, Nelson S (2009) Term labor is associated with a core inflammatory response in human fetal membranes, myometrium, and cervix. Am J Obstet Gynecol 200(104):e1–e11

Bouman A, Heineman MJ, Faas MM (2005) Sex hormones and the immune response in humans. Hum Reprod Update 11:411–423

Bove R, Chitnis T (2014) The role of gender and sex hormones in determining the onset and outcome of multiple sclerosis. Mult Scler 20:520–526

Braundmeier AG, Nowak RA (2006) Cytokines regulate matrix metalloproteinases in human uterine endometrial fibroblast cells through a mechanism that does not involve increases in extracellular matrix metalloproteinase inducer. Am J Reprod Immunol 56:201–214

Bukowski REA (2011) Causes of death among stillbirths. JAMA 306:2459–2468

Bulmer JN, Morrison L, Longfellow M, Ritson A, Pace D (1991) Granulated lymphocytes in human endometrium: histochemical and immunohistochemical studies. Hum Reprod 6: 791–798

Bulmer JN, Williams PJ, Lash GE (2010) Immune cells in the placental bed. Int J Dev Biol 54: 281–294

Caballero-Campo P, Dominguez F, Coloma J, Meseguer M, Remohi J, Pellicer A, Simon C (2002) Hormonal and embryonic regulation of chemokines IL-8, MCP-1 and RANTES in the human endometrium during the window of implantation. Mol Hum Reprod 8:375–384

Care AS, Diener KR, Jasper MJ, Brown HM, Ingman WV, Robertson SA (2013) Macrophages regulate corpus luteum development during embryo implantation in mice. J Clin Invest 123: 3472–3487

Cha J, Sun X, Dey SK (2012) Mechanisms of implantation: strategies for successful pregnancy. Nat Med 18:1754–1767

Challis JR, Lye SJ, Gibb W, Whittle W, Patel F, Alfaidy N (2001) Understanding preterm labor. Ann NY Acad Sci 943:225–234

Chantakru S, Wang WC, Van Den Heuvel M, Bashar S, Simpson A, Chen Q, Croy BA, Evans SS (2003) Coordinate regulation of lymphocyte-endothelial interactions by pregnancy-associated hormones. J Immunol 171:4011–4019

Charnock-Jones DS, Sharkey AM, Fenwick P, Smith SK (1994) Leukaemia inhibitory factor mRNA concentration peaks in human endometrium at the time of implantation and the blastocyst contains mRNA for the receptor at this time. J Reprod Fertil 101:421–426

Chazara O, Xiong S, Moffett A (2011) Maternal KIR and fetal HLA-C: a fine balance. J Leukoc Biol 90:703–716

Chen T, Darrasse-Jeze G, Bergot AS, Courau T, Churlaud G, Valdivia K, Strominger JL, Ruocco MG, Chaouat G, Klatzmann D (2013) Self-specific memory regulatory T cells protect embryos at implantation in mice. J Immunol 191:2273–2281

Chevillard G, Derjuga A, Devost D, Zingg HH, Blank V (2007) Identification of interleukin-1beta regulated genes in uterine smooth muscle cells. Reproduction 134:811–822

Colasanti T, Maselli A, Conti F, Sanchez M, Alessandri C, Barbati C, Vacirca D, Tinari A, Chiarotti F, Giovannetti A, Franconi F, Valesini G, Malorni W, Pierdominici M, Ortona E (2012) Autoantibodies to estrogen receptor alpha interfere with T lymphocyte homeostasis and are associated with disease activity in systemic lupus erythematosus. Arthritis Rheum 64: 778–787

Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T (1998) Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in multiple sclerosis group. N Engl J Med 339:285–291

Crispin JC, Liossis SN, Kis-Toth K, Lieberman LA, Kyttaris VC, Juang YT, Tsokos GC (2010) Pathogenesis of human systemic lupus erythematosus: recent advances. Trends Mol Med 16: 47–57

Crouch SP, Crocker IP, Fletcher J (1995) The effect of pregnancy on polymorphonuclear leukocyte function. J Immunol 155:5436–5443

Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA, Stewart CL (1996) Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. Proc Natl Acad Sci USA 93: 3115–3120

De M, Wood GW (1990) Influence of oestrogen and progesterone on macrophage distribution in the mouse uterus. J Endocrinol 126:417–424

De M, Choudhuri R, Wood GW (1991) Determination of the number and distribution of macrophages, lymphocytes, and granulocytes in the mouse uterus from mating through implantation. J Leukoc Biol 50:252–262

Dimitriadis E, White CA, Jones RL, Salamonsen LA (2005) Cytokines, chemokines and growth factors in endometrium related to implantation. Hum Reprod Update 11:613–630

Disanto G, Ramagopalan SV (2013) On the sex ratio of multiple sclerosis. Mult Scler 19:3–4

Dominguez F, Remohi J, Pellicer A, Simon C (2002) Paracrine interactions during human implantation. Rev Endocr Metab Disord 3:97–105

Dominguez F, Galan A, Martin JJ, Remohi J, Pellicer A, Simon C (2003a) Hormonal and embryonic regulation of chemokine receptors CXCR1, CXCR4, CCR5 and CCR2B in the human endometrium and the human blastocyst. Mol Hum Reprod 9:189–198

Dominguez F, Pellicer A, Simon C (2003b) The chemokine connection: hormonal and embryonic regulation at the human maternal-embryonic interface – a review. Placenta 24(Suppl B): S48–S55

Dosiou C, Giudice LC (2005) Natural killer cells in pregnancy and recurrent pregnancy loss: endocrine and immunologic perspectives. Endocr Rev 26:44–62

Druckmann R, Druckmann MA (2005) Progesterone and the immunology of pregnancy. J Steroid Biochem Mol Biol 97:389–396

Dunn CL, Kelly RW, Critchley HO (2003) Decidualization of the human endometrial stromal cell: an enigmatic transformation. Reprod Biomed Online 7:151–161

Eidinger D, Garrett TJ (1972) Studies of the regulatory effects of the sex hormones on antibody formation and stem cell differentiation. J Exp Med 136:1098–1116

El-Etr M, Vukusic S, Gignoux L, Durand-Dubief F, Achiti I, Baulieu EE, Confavreux C (2005) Steroid hormones in multiple sclerosis. J Neurol Sci 233:49–54

El-Maallem H, Fletcher J (1980) Impaired neutrophil function and myeloperoxidase deficiency in pregnancy. Br J Haematol 44:375–381

Ferry BL, Starkey PM, Sargent IL, Watt GM, Jackson M, Redman CW (1990) Cell populations in the human early pregnancy decidua: natural killer activity and response to interleukin-2 of CD56-positive large granular lymphocytes. Immunology 70:446–452

Foocharoen C, Nanagara R, Salang L, Suwannaroj S, Mahakkanukrauh A (2009) Pregnancy and disease outcome in patients with systemic lupus erythematosus (SLE): a study at Srinagarind Hospital. J Med Assoc Thai 92:167–174

Giltay EJ, Fonk JC, Von Blomberg BM, Drexhage HA, Schalkwijk C, Gooren LJ (2000) In vivo effects of sex steroids on lymphocyte responsiveness and immunoglobulin levels in humans. J Clin Endocrinol Metab 85:1648–1657

Goldenberg RL, Hauth JC, Andrews WW (2000) Intrauterine infection and preterm delivery. N Engl J Med 342:1500–1507

Guerin LR, Prins JR, Robertson SA (2009) Regulatory T-cells and immune tolerance in pregnancy: a new target for infertility treatment? Hum Reprod Update 15:517–535

Guerin LR, Moldenhauer LM, Prins JR, Bromfield JJ, Hayball JD, Robertson SA (2011) Seminal fluid regulates accumulation of FOXP3+ regulatory T cells in the preimplantation mouse uterus through expanding the FOXP3+ cell pool and CCL19-mediated recruitment. Biol Reprod 85: 397–408

Gutierrez G, Gentile T, Miranda S, Margni RA (2005) Asymmetric antibodies: a protective arm in pregnancy. Chem Immunol Allergy 89:158–168

Haines BP, Voyle RB, Rathjen PD (2000) Intracellular and extracellular leukemia inhibitory factor proteins have different cellular activities that are mediated by distinct protein motifs. Mol Biol Cell 11:1369–1383

Hammer A, Hutter H, Blaschitz A, Mahnert W, Hartmann M, Uchanska-Ziegler B, Ziegler A, Dohr G (1997) Amnion epithelial cells, in contrast to trophoblast cells, express all classical HLA class I molecules together with HLA-G. Am J Reprod Immunol 37:161–171

Hampl R, Kubatova J, Heracek J, Sobotka V, Starka L (2013) Hormones and endocrine disruptors in human seminal plasma. Endocr Regul 47:149–158

Handwerger S (1994) A critical role for interleukin-1 (IL-1) and the type 1 IL-1 receptor in blastocyst implantation. Endocrinology 134:519–520

Hannan NJ, Salamonsen LA (2007) Role of chemokines in the endometrium and in embryo implantation. Curr Opin Obstet Gynecol 19:266–272

Hannan NJ, Jones RL, Critchley HO, Kovacs GJ, Rogers PA, Affandi B, Salamonsen LA (2004) Coexpression of fractalkine and its receptor in normal human endometrium and in endometrium from users of progestin-only contraception supports a role for fractalkine in leukocyte recruitment and endometrial remodeling. J Clin Endocrinol Metab 89:6119–6129

Hirota Y, Osuga Y, Koga K, Yoshino O, Hirata T, Morimoto C, Harada M, Takemura Y, Nose E, Yano T, Tsutsumi O, Taketani Y (2006) The expression and possible roles of chemokine CXCL11 and its receptor CXCR3 in the human endometrium. J Immunol 177:8813–8821

Hua R, Pease JE, Sooranna SR, Viney JM, Nelson SM, Myatt L, Bennett PR, Johnson MR (2012) Stretch and inflammatory cytokines drive myometrial chemokine expression via NF-kappaB activation. Endocrinology 153:481–491

Hunt JS, Langat DL (2009) HLA-G: a human pregnancy-related immunomodulator. Curr Opin Pharmacol 9:462–469

Hunt JS, Petroff MG, Mcintire RH, Ober C (2005) HLA-G and immune tolerance in pregnancy. FASEB J 19:681–693

Hviid TV (2006) HLA-G in human reproduction: aspects of genetics, function and pregnancy complications. Hum Reprod Update 12:209–232

Inoue K, Inoue E, Imai Y (2013) Female sex hormones ameliorate arthritis in SKG mice. Biochem Biophys Res Commun 434:740–745

Ishitani A, Sageshima N, Lee N, Dorofeeva N, Hatake K, Marquardt H, Geraghty DE (2003) Protein expression and peptide binding suggest unique and interacting functional roles for HLA-E, F, and G in maternal-placental immune recognition. J Immunol 171:1376–1384

Ito M, Nakashima A, Hidaka T, Okabe M, Bac ND, Ina S, Yoneda S, Shiozaki A, Sumi S, Tsuneyama K, Nikaido T, Saito S (2010) A role for IL-17 in induction of an inflammation at the fetomaternal interface in preterm labour. J Reprod Immunol 84:75–85

Johansson M, Bromfield JJ, Jasper MJ, Robertson SA (2004) Semen activates the female immune response during early pregnancy in mice. Immunology 112:290–300

Jones RL, Hannan NJ, Kaitu'u TJ, Zhang J, Salamonsen LA (2004) Identification of chemokines important for leukocyte recruitment to the human endometrium at the times of embryo implantation and menstruation. J Clin Endocrinol Metab 89:6155–6167

Jones LA, Anthony JP, Henriquez FL, Lyons RE, Nickdel MB, Carter KC, Alexander J, Roberts CW (2008) Toll-like receptor-4-mediated macrophage activation is differentially regulated by progesterone via the glucocorticoid and progesterone receptors. Immunology 125:59–69

Kallikourdis M, Betz AG (2007) Periodic accumulation of regulatory T cells in the uterus: preparation for the implantation of a semi-allogeneic fetus? PLoS One 2:e382

Kim S, Liva SM, Dalal MA, Verity MA, Voskuhl RR (1999) Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. Neurology 52:1230–1238

Kimber SJ (2005) Leukaemia inhibitory factor in implantation and uterine biology. Reproduction 130:131–145

Kindzelskii AL, Ueki T, Michibata H, Chaiworapongsa T, Romero R, Petty HR (2004) 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase form a supramolecular complex in human neutrophils that undergoes retrograde trafficking during pregnancy. J Immunol 172:6373–6381

King A (2000) Uterine leukocytes and decidualization. Hum Reprod Update 6:28–36

King A, Allan DS, Bowen M, Powis SJ, Joseph S, Verma S, Hiby SE, Mcmichael AJ, Loke YW, Braud VM (2000a) HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. Eur J Immunol 30:1623–1631

King A, Burrows TD, Hiby SE, Bowen JM, Joseph S, Verma S, Lim PB, Gardner L, Le Bouteiller P, Ziegler A, Uchanska-Ziegler B, Loke YW (2000b) Surface expression of HLA-C antigen by human extravillous trophoblast. Placenta 21:376–387

Kitaya K, Yasuda J, Yagi I, Tada Y, Fushiki S, Honjo H (2000) IL-15 expression at human endometrium and decidua. Biol Reprod 63:683–687

Kuang H, Peng H, Xu H, Zhang B, Peng J, Tan Y (2010) Hormonal regulation of uterine natural killer cells in mouse preimplantation uterus. J Mol Histol 41:1–7

Kuijper EA, Ket JC, Caanen MR, Lambalk CB (2013) Reproductive hormone concentrations in pregnancy and neonates: a systematic review. Reprod Biomed Online 27:33–63

Labeta MO, Margni RA, Leoni J, Binaghi RA (1986) Structure of asymmetric non-precipitating antibody: presence of a carbohydrate residue in only one Fab region of the molecule. Immunology 57:311–317

Laird SM, Widdowson R, El-Sheikhi M, Hall AJ, Li TC (2011) Expression of CXCL12 and CXCR4 in human endometrium; effects of CXCL12 on MMP production by human endometrial cells. Hum Reprod 26:1144–1152

Lasarte S, Elsner D, Guia-Gonzalez M, Ramos-Medina R, Sanchez-Ramon S, Esponda P, Munoz-Fernandez MA, Relloso M (2013) Female sex hormones regulate the Th17 immune response to sperm and Candida albicans. Hum Reprod 28:3283–3291

Leber A, Teles A, Zenclussen AC (2010) Regulatory T cells and their role in pregnancy. Am J Reprod Immunol 63:445–459

Ledingham MA, Thomson AJ, Jordan F, Young A, Crawford M, Norman JE (2001) Cell adhesion molecule expression in the cervix and myometrium during pregnancy and parturition. Obstet Gynecol 97:235–242

Lee JH, Ulrich B, Cho J, Park J, Kim CH (2011) Progesterone promotes differentiation of human cord blood fetal T cells into T regulatory cells but suppresses their differentiation into Th17 cells. J Immunol 187:1778–1787

Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG (1993) Synthesis of T-helper 2-type cytokines at the maternal-fetal interface. J Immunol 151:4562–4573

Linn S, Murtaugh B, Casey E (2012) Role of sex hormones in the development of osteoarthritis. PM R 4:S169–S173

Liu L, Wang Z (2013) Estrogen attenuates lipopolysaccharide-induced nitric oxide production in macrophages partially via the nongenomic pathway. Cell Immunol 286:53–58

Liu X, Wu H, Byrne M, Jeffrey J, Krane S, Jaenisch R (1995) A targeted mutation at the known collagenase cleavage site in mouse type I collagen impairs tissue remodeling. J Cell Biol 130: 227–237

Liu F, Guo J, Tian T, Wang H, Dong F, Huang H, Dong M (2011) Placental trophoblasts shifted Th1/Th2 balance toward Th2 and inhibited Th17 immunity at fetomaternal interface. APMIS 119:597–604

Loras B, Vetele F, El Malki A, Rollet J, Soufir JC, Benahmed M (1999) Seminal transforming growth factor-beta in normal and infertile men. Hum Reprod 14:1534–1539

Loudon JA, Sooranna SR, Bennett PR, Johnson MR (2004) Mechanical stretch of human uterine smooth muscle cells increases IL-8 mRNA expression and peptide synthesis. Mol Hum Reprod 10:895–899

Loza MJ, Peters SP, Zangrilli JG, Perussia B (2002) Distinction between IL-13+ and IFN-gamma+ natural killer cells and regulation of their pool size by IL-4. Eur J Immunol 32:413–423

Luppi P, Haluszczak C, Betters D, Richard CA, Trucco M, Deloia JA (2002a) Monocytes are progressively activated in the circulation of pregnant women. J Leukoc Biol 72:874–884

Luppi P, Haluszczak C, Trucco M, Deloia JA (2002b) Normal pregnancy is associated with peripheral leukocyte activation. Am J Reprod Immunol 47:72–81

Ma WG, Song H, Das SK, Paria BC, Dey SK (2003) Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. Proc Natl Acad Sci USA 100:2963–2968

Maeda Y, Ohtsuka H, Tomioka M, Oikawa M (2013) Effect of progesterone on Th1/Th2/Th17 and regulatory T cell-related genes in peripheral blood mononuclear cells during pregnancy in cows. Vet Res Commun 37:43–49

Maegawa M, Kamada M, Irahara M, Yamamoto S, Yoshikawa S, Kasai Y, Ohmoto Y, Gima H, Thaler CJ, Aono T (2002) A repertoire of cytokines in human seminal plasma. J Reprod Immunol 54:33–42

Manase K, Endo T, Chida M, Nagasawa K, Honnma H, Yamazaki K, Kitajima Y, Goto T, Kanaya M, Hayashi T, Mitaka T, Saito T (2006) Coordinated elevation of membrane type 1-matrix metalloproteinase and matrix metalloproteinase-2 expression in rat uterus during postpartum involution. Reprod Biol Endocrinol 4:32

Manaster I, Mandelboim O (2010) The unique properties of uterine NK cells. Am J Reprod Immunol 63:434–444

Manaster I, Mizrahi S, Goldman-Wohl D, Sela HY, Stern-Ginossar N, Lankry D, Gruda R, Hurwitz A, Bdolah Y, Haimov-Kochman R, Yagel S, Mandelboim O (2008) Endometrial NK cells are special immature cells that await pregnancy. J Immunol 181:1869–1876

Mao G, Wang J, Kang Y, Tai P, Wen J, Zou Q, Li G, Ouyang H, Xia G, Wang B (2010) Progesterone increases systemic and local uterine proportions of CD4+CD25+ Treg cells during midterm pregnancy in mice. Endocrinology 151:5477–5488

Mariee N, Li TC, Laird SM (2012) Expression of leukaemia inhibitory factor and interleukin 15 in endometrium of women with recurrent implantation failure after IVF; correlation with the number of endometrial natural killer cells. Hum Reprod 27:1946–1954

Matthiesen L, Berg G, Ernerudh J, Skogh T (1995) Lymphocyte subsets and autoantibodies in pregnancies complicated by placental disorders. Am J Reprod Immunol 33:31–39

Medina KL, Kincade PW (1994) Pregnancy-related steroids are potential negative regulators of B lymphopoiesis. Proc Natl Acad Sci USA 91:5382–5386

Menzies FM, Henriquez FL (2009) Immunomodulation by the female sex hormones. Open Infect Dis J 3:61–72

Menzies FM, Henriquez FL, Alexander J, Roberts CW (2011a) Selective inhibition and augmentation of alternative macrophage activation by progesterone. Immunology 134:281–291

Menzies FM, Higgins CA, Shepherd MC, Nibbs RJ, Nelson SM (2011b) Mast cells reside in myometrium and cervix, but are dispensable in mice for successful pregnancy and labor. Immunol Cell Biol 90:321–329

Menzies FM, Khan AH, Higgins CA, Nelson SM, Nibbs RJ (2012) The chemokine receptor CCR2 is not required for successful initiation of labor in mice. Biol Reprod 86:118
Minagawa M, Narita J, Tada T, Maruyama S, Shimizu T, Bannai M, Oya H, Hatakeyama K, Abo T (1999) Mechanisms underlying immunologic states during pregnancy: possible association of the sympathetic nervous system. Cell Immunol 196:1–13
Moffett-King A (2002) Natural killer cells and pregnancy. Nat Rev Immunol 2:656–663
Moldenhauer LM, Diener KR, Thring DM, Brown MP, Hayball JD, Robertson SA (2009) Cross-presentation of male seminal fluid antigens elicits T cell activation to initiate the female immune response to pregnancy. J Immunol 182:8080–8093
Mor G, Cardenas I, Abrahams V, Guller S (2011) Inflammation and pregnancy: the role of the immune system at the implantation site. Ann NY Acad Sci 1221:80–87
Mulayim N, Palter SF, Kayisli UA, Senturk L, Arici A (2003) Chemokine receptor expression in human endometrium. Biol Reprod 68:1491–1495
Mulic-Lutvica A, Bekuretsion M, Bakos O, Axelsson O (2001) Ultrasonic evaluation of the uterus and uterine cavity after normal, vaginal delivery. Ultrasound Obstet Gynecol 18:491–498
Muzzio D, Zenclussen AC, Jensen F (2013) The role of B cells in pregnancy: the good and the bad. Am J Reprod Immunol 69:408–412
Nakashima A, Ito M, Yoneda S, Shiozaki A, Hidaka T, Saito S (2010) Circulating and decidual Th17 cell levels in healthy pregnancy. Am J Reprod Immunol 63:104–109
Nelson JL, Ostensen M (1997) Pregnancy and rheumatoid arthritis. Rheum Dis Clin North Am 23: 195–212
Ng SC, Gilman-Sachs A, Thaker P, Beaman KD, Beer AE, Kwak-Kim J (2002) Expression of intracellular Th1 and Th2 cytokines in women with recurrent spontaneous abortion, implantation failures after IVF/ET or normal pregnancy. Am J Reprod Immunol 48:77–86
Norwitz ER, Schust DJ, Fisher SJ (2001) Implantation and the survival of early pregnancy. N Engl J Med 345:1400–1408
Oertelt-Prigione S (2012) The influence of sex and gender on the immune response. Autoimmun Rev 11:A479–A485
Oh MJ, Croy BA (2008) A map of relationships between uterine natural killer cells and progesterone receptor expressing cells during mouse pregnancy. Placenta 29:317–323
Osman I, Young A, Ledingham MA, Thomson AJ, Jordan F, Greer IA, Norman JE (2003) Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. Mol Hum Reprod 9:41–45
Pennell LM, Galligan CL, Fish EN (2012) Sex affects immunity. J Autoimmun 38:J282–J291
Piccinni MP, Giudizi MG, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, Parronchi P, Manetti R, Annunziato F, Livi C et al (1995) Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. J Immunol 155:128–133
Piccinni MP, Beloni L, Livi C, Maggi E, Scarselli G, Romagnani S (1998) Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. Nat Med 4:1020–1024
Poehlmann TG, Fitzgerald JS, Meissner A, Wengenmayer T, Schleussner E, Friedrich K, Markert UR (2005) Trophoblast invasion: tuning through LIF, signalling via Stat3. Placenta 26(Suppl A):S37–S41
Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, Offner H (2004) Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. J Immunol 173:2227–2230
Quinn KH, Lacoursiere DY, Cui L, Bui J, Parast MM (2011) The unique pathophysiology of early-onset severe preeclampsia: role of decidual T regulatory cells. J Reprod Immunol 91:76–82
Robb L, Dimitriadis E, Li R, Salamonsen LA (2002) Leukemia inhibitory factor and interleukin-11: cytokines with key roles in implantation. J Reprod Immunol 57:129–141
Robertson SA (2005) Seminal plasma and male factor signalling in the female reproductive tract. Cell Tissue Res 322:43–52

Robertson SA, O'Connell A, Ramsey A (2000) The effect of interleukin-6 deficiency on implantation, fetal development and parturition in mice. Proc Aust Soc Reprod Biol 31:97
Robertson SA, Guerin LR, Bromfield JJ, Branson KM, Ahlstrom AC, Care AS (2009a) Seminal fluid drives expansion of the CD4+CD25+ T regulatory cell pool and induces tolerance to paternal alloantigens in mice. Biol Reprod 80:1036–1045
Robertson SA, Guerin LR, Moldenhauer LM, Hayball JD (2009b) Activating T regulatory cells for tolerance in early pregnancy – the contribution of seminal fluid. J Reprod Immunol 83:109–116
Rossant J, Cross JC (2001) Placental development: lessons from mouse mutants. Nat Rev Genet 2:538–548
Rowe JH, Ertelt JM, Xin L, Way SS (2012) Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. Nature 490:102–106
Sadowska A (2005) Pregnancy in women with systemic lupus erythematosus (SLE). Przegl Lek 62:61–64
Saito S (2000) Cytokine network at the feto-maternal interface. J Reprod Immunol 47:87–103
Saito S, Nakashima A, Shima T, Ito M (2010) Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol 63:601–610
Salamonsen LA (2003) Tissue injury and repair in the female human reproductive tract. Reproduction 125:301–311
Salker MS, Nautiyal J, Steel JH, Webster Z, Sucurovic S, Nicou M, Singh Y, Lucas ES, Murakami K, Chan YW, James S, Abdallah Y, Christian M, Croy BA, Mulac-Jericevic B, Quenby S, Brosens JJ (2012) Disordered IL-33/ST2 activation in decidualizing stromal cells prolongs uterine receptivity in women with recurrent pregnancy loss. PLoS One 7:e52252
Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, Fazekas De St Groth B, Nanan R (2009) Systemic increase in the ratio between Foxp3+ and IL-17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. J Immunol 183:7023–7030
Scaife PJ, Bulmer JN, Robson SC, Innes BA, Searle RF (2006) Effector activity of decidual CD8+ T lymphocytes in early human pregnancy. Biol Reprod 75:562–567
Sharkey DJ, Macpherson AM, Tremellen KP, Mottershead DG, Gilchrist RB, Robertson SA (2012a) TGF-beta mediates proinflammatory seminal fluid signaling in human cervical epithelial cells. J Immunol 189:1024–1035
Sharkey DJ, Tremellen KP, Jasper MJ, Gemzell-Danielsson K, Robertson SA (2012b) Seminal fluid induces leukocyte recruitment and cytokine and chemokine mRNA expression in the human cervix after coitus. J Immunol 188:2445–2454
Shobu T, Sageshima N, Tokui H, Omura M, Saito K, Nagatsuka Y, Nakanishi M, Hayashi Y, Hatake K, Ishitani A (2006) The surface expression of HLA-F on decidual trophoblasts increases from mid to term gestation. J Reprod Immunol 72:18–32
Shynlova O, Tsui P, Dorogin A, Lye SJ (2008) Monocyte chemoattractant protein-1 (CCL-2) integrates mechanical and endocrine signals that mediate term and preterm labor. J Immunol 181:1470–1479
Shynlova O, Lee YH, Srikhajon K, Lye S (2012) Physiologic uterine inflammation and labor onset: integration of endocrine and mechanical signals. Reprod Sci 20:154–167
Simon C, Pellicer A, Polan ML (1995) Interleukin-1 system crosstalk between embryo and endometrium in implantation. Hum Reprod 10(Suppl 2):43–54
Simon C, Mercader A, Gimeno MJ, Pellicer A (1997) The interleukin-1 system and human implantation. Am J Reprod Immunol 37:64–72
Skurupiy VA, Obedinskaya KS, Nadeev AP (2010) Structural manifestations of mechanisms of myometrium involution after repeated pregnancies in mice. Bull Exp Biol Med 149:554–558
Smith R (2007) Parturition. N Engl J Med 356:271–283
Smith GC, Crossley JA, Aitken DA, Pell JP, Cameron AD, Connor JM, Dobbie R (2004) First-trimester placentation and the risk of antepartum stillbirth. JAMA 292:2249–2254
Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT (2004) Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. Immunology 112:38–43

Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F, Abbondanzo SJ (1992) Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 359:76–79

Suman P, Malhotra SS, Gupta SK (2013) LIF-STAT signaling and trophoblast biology. JAKSTAT 2:e25155

Szekeres-Bartho J, Wegmann TG (1996) A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. J Reprod Immunol 31:81–95

Szekeres-Bartho J, Par G, Dombay G, Smart YC, Volgyi Z (1997) The antiabortive effect of progesterone-induced blocking factor in mice is manifested by modulating NK activity. Cell Immunol 177:194–199

Tabibzadeh S, Kong QF, Babaknia A, May LT (1995) Progressive rise in the expression of interleukin-6 in human endometrium during menstrual cycle is initiated during the implantation window. Hum Reprod 10:2793–2799

Tang AW, Alfirevic Z, Quenby S (2011) Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: a systematic review. Hum Reprod 26:1971–1980

Teles A, Zenclussen AC, Schumacher A (2013) Regulatory T cells are baby's best friends. Am J Reprod Immunol 69:331–339

Thomson AJ, Telfer JF, Young A, Campbell S, Stewart CJ, Cameron IT, Greer IA, Norman JE (1999) Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. Hum Reprod 14:229–236

Thuere C, Zenclussen ML, Schumacher A, Langwisch S, Schulte-Wrede U, Teles A, Paeschke S, Volk HD, Zenclussen AC (2007) Kinetics of regulatory T cells during murine pregnancy. Am J Reprod Immunol 58:514–523

Tilburgs T, Strominger JL (2013) CD8+ effector T cells at the fetal-maternal interface, balancing fetal tolerance and antiviral immunity. Am J Reprod Immunol 69:395–407

Tilburgs T, Roelen DL, Van Der Mast BJ, Van Schip JJ, Kleijburg C, De Groot-Swings GM, Kanhai HH, Claas FH, Scherjon SA (2006) Differential distribution of CD4(+)CD25(bright) and CD8(+)CD28(−) T-cells in decidua and maternal blood during human pregnancy. Placenta 27(Suppl A):S47–S53

Tilburgs T, Roelen DL, Van Der Mast BJ, De Groot-Swings GM, Kleijburg C, Scherjon SA, Claas FH (2008) Evidence for a selective migration of fetus-specific CD4+CD25bright regulatory T cells from the peripheral blood to the decidua in human pregnancy. J Immunol 180:5737–5745

Tilburgs T, Schonkeren D, Eikmans M, Nagtzaam NM, Datema G, Swings GM, Prins F, Van Lith JM, Van Der Mast BJ, Roelen DL, Scherjon SA, Claas FH (2010) Human decidual tissue contains differentiated CD8+ effector-memory T cells with unique properties. J Immunol 185: 4470–4477

Timmons BC, Fairhurst AM, Mahendroo MS (2009) Temporal changes in myeloid cells in the cervix during pregnancy and parturition. J Immunol 182:2700–2707

Tremellen KP, Seamark RF, Robertson SA (1998) Seminal transforming growth factor beta1 stimulates granulocyte-macrophage colony-stimulating factor production and inflammatory cell recruitment in the murine uterus. Biol Reprod 58:1217–1225

Tremellen KP, Valbuena D, Landeras J, Ballesteros A, Martinez J, Mendoza S, Norman RJ, Robertson SA, Simon C (2000) The effect of intercourse on pregnancy rates during assisted human reproduction. Hum Reprod 15:2653–2658

Vacca P, Vitale C, Montaldo E, Conte R, Cantoni C, Fulcheri E, Darretta V, Moretta L, Mingari MC (2011) CD34+ hematopoietic precursors are present in human decidua and differentiate into natural killer cells upon interaction with stromal cells. Proc Natl Acad Sci USA 108: 2402–2407

Vacca P, Mingari MC, Moretta L (2013) Natural killer cells in human pregnancy. J Reprod Immunol 97:14–19

Wang WJ, Hao CF, Yi L, Yin GJ, Bao SH, Qiu LH, Lin QD (2010) Increased prevalence of T helper 17 (Th17) cells in peripheral blood and decidua in unexplained recurrent spontaneous abortion patients. J Reprod Immunol 84:164–170

Wegmann TG, Lin H, Guilbert L, Mosmann TR (1993) Bidirectional cytokine interactions in the maternal-fetal relationship – is successful pregnancy a Th2 phenomenon. Immunol Today 14: 353–356

Wilcox AJ, Weinberg CR, O'connor JF, Baird DD, Schlatterer JP, Canfield RE, Armstrong EG, Nisula BC (1988) Incidence of early loss of pregnancy. N Engl J Med 319:189–194

Wilcox AJ, Baird DD, Weinberg CR (1999) Time of implantation of the conceptus and loss of pregnancy. N Engl J Med 340:1796–1799

Winkler M, Kemp B, Fischer DC, Ruck P, Rath W (2003) Expression of adhesion molecules in the lower uterine segment during term and preterm parturition. Microsc Res Tech 60:430–444

Woessner JF Jr (1996) Regulation of matrilysin in the rat uterus. Biochem Cell Biol 74:777–784

Yan Yuen S, Krizova A, Ouimet JM, Pope JE (2008) Pregnancy outcome in systemic lupus erythematosus (SLE) is improving: results from a case control study and literature review. Open Rheumatol J 2:89–98

Yang Y, Chu W, Geraghty DE, Hunt JS (1996) Expression of HLA-G in human mononuclear phagocytes and selective induction by IFN-gamma. J Immunol 156:4224–4231

Yeaman GR, Guyre PM, Fanger MW, Collins JE, White HD, Rathbun W, Orndorff KA, Gonzalez J, Stern JE, Wira CR (1997) Unique CD8+ T cell-rich lymphoid aggregates in human uterine endometrium. J Leukoc Biol 61:427–435

Yeaman GR, Collins JE, Fanger MW, Wira CR, Lydyard PM (2001) CD8+ T cells in human uterine endometrial lymphoid aggregates: evidence for accumulation of cells by trafficking. Immunology 102:434–440

Young A, Thomson AJ, Ledingham M, Jordan F, Greer IA, Norman JE (2002) Immunolocalization of proinflammatory cytokines in myometrium, cervix, and fetal membranes during human parturition at term. Biol Reprod 66:445–449

Zenclussen AC, Gentile T, Kortebani G, Mazzolli A, Margni R (2001) Asymmetric antibodies and pregnancy. Am J Reprod Immunol 45:289–294

Zenclussen ML, Thuere C, Ahmad N, Wafula PO, Fest S, Teles A, Leber A, Casalis PA, Bechmann I, Priller J, Volk HD, Zenclussen AC (2010) The persistence of paternal antigens in the maternal body is involved in regulatory T-cell expansion and fetal-maternal tolerance in murine pregnancy. Am J Reprod Immunol 63:200–208

Zhang J, Lathbury LJ, Salamonsen LA (2000) Expression of the chemokine eotaxin and its receptor, CCR3, in human endometrium. Biol Reprod 62:404–411

Zinaman MJ, Clegg ED, Brown CC, O'connor J, Selevan SG (1996) Estimates of human fertility and pregnancy loss. Fertil Steril 65:503–509

Chapter 4
Sex Differences in Metabolism and Pharmacokinetics

Anandi N. Sheth, Cecile D. Lahiri, and Ighovwerha Ofotokun

Abstract Men and women may differ in their therapeutic and/or adverse responses to certain drugs. These differences can be due to sex- or sex hormone-related effects on the drug disposition process, particularly those involving drug-metabolizing enzymes and transporters. This chapter will review sex-associated differences in drug pharmacokinetics in general, but also focus on the special circumstances of pregnancy, menopause, and the use of hormonal contraceptives, highlighting evidence demonstrating these differences for anti-infectives. Better characterization of the impact of sex on drug dispositions would guide therapeutic choices and dosing schedule for men and women and enhance treatment outcomes for many commonly prescribed anti-infectives.

4.1 Overview: Sex and Drug Disposition

Previous evidence has shown that men and women may differ in their response to certain drugs in terms of therapeutic effect and/or toxicity. This finding is likely to be related to differences in drug absorption and disposition based on sex. Drug disposition refers to what happens to the drug after it enters the body and is affected by drug-metabolizing enzymes and membrane drug transporters. Increasingly, sex hormones have been recognized as affecting drug pharmacokinetics and pharmacodynamics, thereby explaining in part sex differences in response to drugs. Among anti-infectives, such sex differences in drug disposition may be particularly relevant for drugs that require long-term therapy, such as antiretroviral drugs used in HIV therapy.

Recent efforts have focused on the inclusion of women in clinical trials of anti-infectives to better understand sex-related differences in response to these drugs. Clinical data suggest that sex-related pharmacological differences affect both the

A.N. Sheth (✉) • C.D. Lahiri • I. Ofotokun
Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA
e-mail: ansheth@emory.edu

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_4

risk and the rate of adverse drug reactions as well as drug efficacy (Nicolson et al. 2010). For example, with regard to drug toxicity, emerging evidence has shown that women have an increased propensity for developing drug-induced long QT syndrome, a cardiac electrophysiologic disorder (Hreiche et al. 2008). Furthermore, their susceptibility to developing this condition is more prominent during the ovulatory phase of the menstrual cycle, supporting the hypothesis of hormonal influence (Rodriguez et al. 2001). Some clinical trials have suggested that sex-related differences exist in the safety and efficacy of antiretroviral drugs such as those used to treat HIV. Women have higher rates of adverse drug reactions with certain antiretroviral medications compared to men (Ofotokun 2005), such as increased risk of developing a rash with the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine (Mazhude et al. 2002) and increased rates of dyslipidemia and nausea with protease inhibitors (PIs) (Pernerstorfer-Schoen et al. 2001; Squires et al. 2011). There might also be a discrepancy in drug efficacy of some antiretrovirals between men and women; in a prospective cohort of HIV-infected individuals, women achieved virologic suppression at faster rates than men using comparable antiretroviral regimens (Moore et al. 2001).

The antiretroviral data showing increased risk of drug toxicity and a faster rate of virological suppression in women suggest that women may attain higher serum or tissue concentrations of drugs than men due to inherent sex-related differences in pharmacokinetics. Higher intracellular concentrations of the diphosphate and the monophosphate active forms of zidovudine and lamivudine, two nucleoside reverse transcriptase inhibitors (NRTIs), have been observed in women compared to men. These increases in intracellular drug levels resulted in a matched effect in pharmacodynamics as women suppressed their viral load twice as fast as men (Anderson et al. 2003). Additionally, a population pharmacokinetic study on nevirapine found that women had lower drug clearance rates than men (Zhou et al. 1999), which could explain their increased risk of developing a rash.

Mechanisms of sex-related pharmacologic differences in antiretrovirals and other antimicrobials remain incompletely understood and are likely to be multifactorial. Proposed mechanisms include physiologic differences between the sexes (such as total body weight, fat distribution, degree of plasma protein binding, and glomerular filtration rates), the influence of sex hormones on drug metabolism, and sex-related variations of expression and activity of drug transporter genes and proteins (Ofotokun et al. 2007b). Potential reasons for sex differences in adverse events related to drug pharmacokinetics and pharmacodynamics are listed in Table 4.1.

This chapter will review sex-associated differences in pharmacokinetics, including their effects on drug transporters, and focus on the special circumstances of pregnancy, menopause, and the use of hormonal contraceptives. While sex- or sex hormone-based differences have been observed in pharmacokinetic parameters, drug transporters, and clinical effect/toxicity for several classes of drugs, we will focus on the evidence demonstrating these differences for anti-infectives.

Table 4.1 Potential reasons for sex-related differences in drug adverse events

Reason for sex difference	Pharmacological reason	Pharmacological factors
Women are overdosed	Pharmacokinetics	Sex differences in volume of distribution Sex differences in protein binding Sex differences in transport, phase I, and phase II metabolism
Women are more sensitive to drug effects	Pharmacodynamics	Sex differences in drug targets (1) Receptor number (2) Receptor binding (3) Signal transduction following receptor binding
Women are prescribed multiple medications	Drug-drug interactions	Drug-drug-induced alterations in pharmacokinetics and/or pharmacodynamics

Adapted with permission Soldin et al. (2011)

4.2 Impact of Sex on Pharmacokinetics

The term "pharmacokinetics" describes the interaction between a drug and the human body in terms of four major factors—absorption, distribution, metabolism, and elimination. The impact of sex on these four major factors has been the subject of previous reviews (Gandhi et al. 2004; Soldin and Mattison 2009). We will first summarize the main pharmacokinetic parameters that are used to describe absorption, distribution, metabolism, and elimination and then review the contribution of these four factors to sex-related variations in pharmacokinetics (Table 4.2).

4.2.1 Summary of Pharmacokinetic Parameters

Oral Bioavailability (F): Oral bioavailability is the fraction of dose administered that is absorbed into the systemic circulation. It is a function of the fraction of dose absorbed by enterocytes and the fractions that pass the gut membranes and liver intact (Shugarts and Benet 2009).

Absorption Half-Life ($t_{1/2,abs}$): Absorption half-life is the time taken for absorption to be 50 % complete and is inversely proportional to K_a, the rate constant of absorption.

Volume of Distribution (V): The volume of distribution is an apparent volume that relates the total amount of drug in the body divided by the plasma concentration. For orally administered drugs, V cannot be separated from F and is denoted as V/F.

Area Under the Concentration-Time Curve (AUC): The AUC is the integral of the plot of concentration of a drug against time. It is proportional to the oral bioavailability and is a measurement of drug exposure.

Table 4.2 Effect of sex or sex hormones on pharmacokinetic parameters

PK parameter	Components	Sex-based differences	References
Bioavailability	Passive component: gastrointestinal tract physiology	Gastric emptying time is slower in females than males, mainly secondary to the effects of estrogen	Coskun et al. (1995), Hutson et al. (1989)
	Active component: extrusion by intestinal drug transporters	Intestinal p-gp levels do not consistently seem to vary by sex	
	Gut metabolism: gut enzymes and intestinal CYP3A4	Gastric levels of alcohol dehydrogenase are higher in males than females; intestinal CYP3A4 levels do not consistently vary by sex	
Distribution	Body composition: BMI, percent body fat, plasma volume, and organ blood flow	Women have lower body weights and lower BMI than men; women have a higher proportion of body fat than men; plasma volume is greater in men than women, although volume varies throughout the menstrual cycle and during pregnancy; organ blood flow is greater in women than men	Gandhi et al. (2004), Soldin et al. (2011)
	Protein binding: extent of tissue and protein binding of the drug	Albumin concentrations do not consistently vary by sex, but endogenous estrogens decrease levels of AAG in the plasma, so women have lower concentrations of AAG than men. Exogenous estrogens increase levels of the serum-binding globulins	Kishino et al. (2002), Succari et al. (1990), Walle et al. (1994) and Wiegratz et al. (2003ab)
Metabolism	Hepatic enzymes: phase I metabolism reactions in the liver mediated through the cytochrome P450 system	Data on varying levels of CYP expression and activity exist, but the overall studies that examine CYP (mainly CYP3A4) substrates for differences in pharmacokinetic parameters in men and women are inconsistent; general trend suggests higher rates of metabolism for	Waxman and Holloway (2009), Austin et al. (1980)

(continued)

Table 4.2 (continued)

PK parameter	Components	Sex-based differences	References
		CYP3A4 substrates in women versus men	
	Hepatic transporters: hepatic p-gp or MDRI	Men seem to have higher hepatic p-gp levels than women, with higher rates of drug clearance in women versus men for drugs that are p-gp substrates	Schuetz et al. (1995)
Excretion	Renal clearance: renal excretion is depending on filtration, secretion, and reabsorption	Renal clearance of drugs that are not actively secreted or reabsorbed is dependent on GFR, which is proportional to weight; sex differences for these drugs are attributable to weight differences. Drugs that are actively secreted by the kidney may show sex differences in excretion	Austin et al. (1980), Gandhi et al. (2004), Soldin and Mattison (2009)

Adapted with permission Gandhi et al. (2004)
BMI body mass index, *p-gp* p-glycoprotein, *CYP* cytochrome P450, *AAG* alpha-1 acid glycoprotein, *MDRI* multi drug resistance transporter-1, *GFR* glomerular filtration rate

Clearance (CL): Clearance is the volume of body fluid from which a drug is completely removed per unit time. It relates the rate of drug elimination and the drug's concentration.

Elimination Half-Life ($t_{1/2}$): Elimination half-life is the time taken for 50 % of the absorbed dose of a drug to be removed or the time taken for the maximum plasma drug concentration to decrease by 50 %. It is directly proportional to volume of distribution (V) and inversely proportional to CL (Grover and Benet 2009).

4.2.2 Absorption

Drugs may be administered through several routes, including via oral, intradermal, intramuscular, intravenous, rectal, vaginal, intrathecal, and intraperitoneal routes. For orally administered drugs, the rate and extent of drug absorption from the various sites along the gastrointestinal tract directly impact a drug's bioavailability and are affected by food interactions, gastric acid levels, gastric emptying, gut motility, biliary secretion and composition, enteric flora, and drug-specific factors (such as lipid solubility, molecular weight, pH, etc.). Despite sex-related differences in some of these factors discussed below, available studies have only

demonstrated a few drugs that show differences in absorption based on sex (Gandhi et al. 2004).

The impact of sex-related differences in drug absorption on bioavailability remains unclear. The United States Food and Drug Administration (FDA) previously evaluated sex differences in bioequivalence among 26 small ($\leq$20 participants) studies involving participants of both sexes (none involving anti-infectives) submitted between 1977 and 1995 and found that maximum concentration (C_{max}) and AUC were greater in women compared with men 87 and 71 % of the time, respectively (Chen et al. 2000; Soldin et al. 2011). Among anti-infectives, the antituberculous agent, rifampin, has been previously demonstrated to have a higher plasma and urinary concentration in adult women than men after the same oral dose, suggesting an increase in drug absorption and therefore higher bioavailability in women (Iwainsky et al. 1976).

Gastrointestinal Physiology: Some reports suggest that women have less acidic gastric fluid (Collen et al. 1994; Soldin and Mattison 2009), thereby affecting the level of absorption of drugs that have pH-dependent gastric absorption. Despite this, steady-state pharmacokinetic parameters do not differ between men and women for the antiretroviral drug atazanavir (von Hentig et al. 2008), which is known to have gastric acid-dependent absorption. In animal studies, estrogens and high concentrations of progesterone inhibit gastric emptying (Coskun et al. 1995), while low concentrations of progesterone increase gastric emptying (Liu et al. 2002). Gastric emptying is slower in premenopausal women and postmenopausal women receiving hormone replacement therapy compared with men (Hutson et al. 1989). Animal studies also suggest that gut motility may be impacted by sex hormones (Chen et al. 1995) and thus may vary depending on pregnancy, contraception, phase of the reproductive cycle, or menopause. Longer gut transit times, on average, have been demonstrated in women compared with men (Degen and Phillips 1996). In spite of the implied hormonal influence of the above studies, gut transit times do not vary over the menstrual cycle in women (Kamm et al. 1989).

Transporter Proteins: Sex-based variability in drug absorption may also be impacted by sex- or sex hormone-related alterations in the expression of intestinal drug transporters, which are involved in transport of drugs across the gut and are discussed in detail in Sect. 4.3.

Gastrointestinal Enzymes that Metabolize Drugs: Gastric and intestinal expressions of metabolic enzymes, such as gastric alcohol dehydrogenase and intestinal cytochrome P450 (CYP) 3A4, affect first-pass metabolism of some oral drugs and may vary by sex resulting in sex-related differences in plasma concentrations of some drugs. Drug metabolism is discussed further in Sect. 4.2.4.

Drugs Administered via Other (Non-oral) Routes: Although clinical data are not available, men and women might theoretically have different absorption of transdermally administered drugs due to differences in body fat composition (Soldin and Mattison 2009). One study that used modeling estimated less drug disposition of the antiviral drug ribavirin in the respiratory tract by body weight after inhaled dosing in adolescent and young adult women (age $\leq$25years) compared with similar-aged males due to lower basal energy requirements resulting in lower respiratory gas

exchange (Knight et al. 1988). However, data are not available on whether this phenomenon is of clinical significance for ribavirin or other inhaled drugs.

4.2.3 Distribution

Once a drug is absorbed, it is distributed into tissues including its target site. The rate and extent of distribution is affected by multiple factors, including body composition parameters, plasma and total blood volumes, organ blood flow, and the levels of plasma and/or tissue protein capable of binding the drug. Sex-related differences in any of these factors might result in varying drug concentrations at its target site, thereby affecting the clinical effect and/or toxicity of the drug.

Body Composition Differences: On average, women have a higher body fat percentage, lower body weight, lower plasma volume, and lower total body water than men (Gandhi et al. 2004). Due to these differences in plasma volume and total body water, the volume of distribution will be relatively lower in women for water-soluble drugs, resulting in higher drug concentrations. On the other hand, due to differences in body fat percentage, the volume of distribution may be relatively higher in women for lipophilic drugs. With regard to anti-infectives, metronidazole, a water-soluble drug used to treat infections due to anaerobic bacteria and certain parasites, has been found to have a slightly (12 %) lower AUC in women than men with a higher apparent volume of distribution (Carcas et al. 2001). Similarly, ofloxacin and fleroxacin, members of the water-soluble fluoroquinolone class that are commonly used broad-spectrum antibacterial drugs, both demonstrated a smaller median volume of distribution in women than in men (Bertino and Nafziger 1996; Sowinski et al. 1999).

Studies have also demonstrated that, with correction of dose by body weight, some sex-related differences in pharmacokinetic parameters are eliminated, reinforcing the idea that body weight differences, rather than other sex-specific differences, may result in variations in drug disposition. For example, women exhibited higher plasma concentrations of the antibacterial drug clindamycin than men in one study after an oral 600 mg dose, but these concentrations were similar when normalized for body weight (del Carmen Carrasco-Portugal et al. 2008). A similar study demonstrated that after a single oral 200 mg dose of fluconazole, a commonly used antifungal drug, in healthy Chinese adults, sex-related differences in AUC, C_{max}, and volume of distribution which directly correlated with body weight were observed (Guo et al. 2010).

Organ Blood Flow: Although cardiac output, when standardized by body surface area as the cardiac index, does not differ between men and women, organ-specific differences in blood flow as a percentage of cardiac output have been noted between the sexes. For instance, men generally have greater blood flow to skeletal muscle, while women have greater blood flow to adipose tissue, potentially related to sex differences in the total body mass represented by each type of tissue (Soldin et al. 2011). For some organs, such as adrenal glands, bone, brain, lungs, skin,

and thyroid, regional blood flow does not differ by sex. However, for adipose tissue, heart, and liver, regional blood flow is higher for women, while for skeletal muscle and kidneys, regional blood flow is higher for men. This is likely to reflect sex differences in body composition and the contribution of each tissue type to total body mass (Soldin et al. 2011).

Protein Binding: Plasma- and tissue-binding proteins affect the levels of a drug's free (or active) form and may account for sex-related differences in drug distribution. Plasma-binding proteins include albumin, alpha-1 acid glycoprotein (AAG), and alpha globulins. Albumin generally binds acidic drugs with a high capacity, while AAG generally binds basic or neutral drugs with a high affinity. While albumin concentrations have not been found to vary by sex, plasma AAG concentrations are lower in women compared to men as is the degree of AAG glycosylation, which is inversely related to its binding capacity (Kishino et al. 2002). Endogenous and exogenous sex steroid hormones also affect AAG concentrations and the extent of glycosylation (Succari et al. 1990; Walle et al. 1994), as does hormonal contraception, estrogen replacement therapy, and pregnancy (see Sect. 4.4). Additionally, some exogenous sex steroid hormones also increase the levels of the serum-binding globulins, such as sex hormone-binding globulin, corticosteroid-binding globulin, and thyroxine-binding globulin (Wiegratz et al. 2003a, b).

Most studies assessing sex-related differences in plasma and/or tissue concentrations of anti-infectives have measured only total rather than free drug levels and thereby limit interpretation of potential sex-related differences on the concentrations of active drug. HIV PIs, for example, are generally highly, but variably, bound to AAG, thereby affecting the unbound (or active) concentration of the drug in plasma and tissues (Delille et al. 2014). Some studies have noted higher plasma concentrations of certain PIs in women compared with men (Pai et al. 2004; Fletcher et al. 2004), but not others (Ofotokun et al. 2007a). However, one study noted that changes in plasma AAG levels altered total but not free concentrations or the antiviral activity of the PI lopinavir (Ofotokun et al. 2011), suggesting that sex-related changes in AAG may not affect a drug's pharmacodynamics effect.

4.2.4 Metabolism

Sex-related differences in pharmacokinetics of drugs may predominantly result from differences in drug metabolism. Drug metabolism occurs in hepatic and, to a lesser degree, extrahepatic sites, and the rate of metabolism is affected by many drug-specific factors, such as lipophilicity and protein binding. Metabolism (mostly hepatic) occurs in two enzymatic reactions termed phase I and phase II. Phase I, which includes oxidation, reduction, and hydrolysis, is mediated through the CYP P450 system for 95 % of drugs (Wrighton and Stevens 1992). Phase II prepares drugs and phase I metabolic products for excretion by conjugation reactions including glucuronidation, sulfation, acetylation, methylation, and glutathione

Table 4.3 Route of metabolism for various anti-infectives and sex differences in hepatic clearance

Metabolic enzyme	Select anti-infective substrates, inhibitors, or inducers of enzyme	Effect of sex or sex hormones on enzymes activity	References
Phase I enzymes			
CYP3A4	*Substrates*: clarithromycin, clindamycin, dapsone, erythromycin, fluconazole (minor), itraconazole, ketoconazole Mefloquine, quinine, voriconazole (minor), All NNRTIs, All HIV protease inhibitors, cobicistat (major), dolutegravir (minor), elvitegravir Maraviroc, boceprevir, simeprevir, sofosbuvir, telaprevir *Inhibitors*: Azole antifungals, boceprevir, clarithromycin, efavirenz, erythromycin, most HIV protease inhibitors, ritonavir, telaprevir *Inducers*: All NNRTIs, rifampin, tipranavir	F > M[a]	Austin et al. (1980), Cummins et al. (2002), Gorski et al. (1998, 2003), Kang et al. (2003), Kashuba et al. (1998), Krecic-Shepard et al. (2000a, b)
CYP2D6	*Substrates*: cobicistat (minor), darunavir, ritonavir *Inhibitors*: cobicistat, darunavir, indinavir, lopinavir/ritonavir, quinidine, ritonavir, terbinafine, tipranavir *Inducers*: rifampin	F > M	Hagg et al. (2001)
CYP1A2	*Substrates*: rilpivirine *Inhibitors*: ciprofloxacin *Inducers*: nafcillin, rifampin, rilpivirine, ritonavir, tipranavir	M > F	Ou-Yang et al. (2000), Relling et al. (1992)
CYP2C9	*Substrates*: etravirine, nelfinavir, rilpivirine (minor), voriconazole *Inhibitors*: efavirenz, fluconazole, isoniazid, itraconazole, metronidazole, ritonavir, sulfamethoxazole,	Unknown	–

(continued)

Table 4.3 (continued)

Metabolic enzyme	Select anti-infective substrates, inhibitors, or inducers of enzyme	Effect of sex or sex hormones on enzymes activity	References
	trimethoprim, voriconazole *Inducers*: darunavir, elvitegravir, indinavir, lopinavir/ritonavir, nelfinavir, rifampin, ritonavir, tipranavir		
CYP2C19	*Substrates*: chloramphenicol, etravirine, nelfinavir, rilpivirine, voriconazole (major) *Inhibitors*: chloramphenicol, efavirenz, etravirine, isoniazid, ketoconazole, ritonavir, voriconazole *Inducers*: darunavir, indinavir, lopinavir/ritonavir, nelfinavir, rifampin, rilpivirine, ritonavir, tipranavir	M = F	Laine et al. (2000)
CYP2E1	*Substrates*: clarithromycin *Inhibitors*: ritonavir *Inducers*: isoniazid	M > F	Kim and O'Shea (1995), Lucas et al. (1995)
Phase II enzymes			
UDP-glucuronosyl transferases	*Substrates*: dolutegravir, elvitegravir, raltegravir, voriconazole *Inhibitors*: atazanavir *Inducers*: efavirenz, rifampin, ritonavir, tipranavir	M > F	Boudikova et al. (1990), Court et al. (2001), Miners et al. (1983), Morissette et al. (2001)
Sulfotransferases		M > F	Brittelli et al. (1999)
N-acetyl-transferases	*Substrates*: sulfamethoxazole	Unknown	–
Methyl-transferases		M > F	Szumlanski et al. (1992), Boudikova et al. (1990)

CYP cytochrome P450 isoenzyme, *M > F* greater enzyme activity in males than females, M = F no evidence of sex-related differences in enzyme activity, *F > M* greater enzyme activity in females than males, *UDP* uridine 5-diphosphate glucuronosyltransferase

[a]Data are conflicting (see text)

conjugation. Intestinal cells also express phase I (CYP3A) and II enzymes, contributing to first-pass metabolism for some orally administered drugs. The rate of hepatic metabolism is affected by hepatic blood flow (which is lower for women) and hepatic enzyme activity, but it is thought that sex differences in enzyme activity, presumably mediated by the effect of endogenous sex hormones on enzyme expression, account for related differences in pharmacokinetics (Waxman and Holloway 2009). Table 4.3 summarizes known studies on sex-related differences in phase I and phase II enzymatic activity.

Phase I Enzymes: The CYP450 superfamily of enzymes consists of at least 11 different families, of which CYP 1, 2, and 3 are most important in humans. In humans, CYP3A is the predominant CYP450 isoenzyme in the liver and is responsible for the metabolism of nearly one half of all drugs that undergo phase I metabolism, including many anti-infectives. Additionally, antimicrobials such as azole antifungals, macrolide antibiotics, and rifampin may either induce or inhibit activity of CYP450 enzymes, thereby impacting the exposure of drugs metabolized by these enzymes (Table 4.3). Among antiretrovirals, NRTIs do not exhibit hepatic metabolism, while NNRTIs, PIs, some integrase strand transfer inhibitors, and the entry inhibitor maraviroc are CYP3A substrates. Ritonavir and cobicistat inhibit CYP3A4, while some PIs and NNRTIs induce CYP3A.

Differences in CYP450 enzymes between the sexes have been noted in previous studies and are affected by endogenous sex hormones (Kashuba and Nafziger 1998) and hormonal changes associated with hormonal contraceptives, pregnancy, and menopause (see Sect. 4.4). Studies of sex-related differences in CYP450 activity have assessed CYP enzyme mRNA expression, CYP activity, or pharmacokinetic parameters of CYP-metabolized drugs in vitro or in animal models and human studies (reviewed, Gandhi et al. 2004). We will summarize below the data from human studies assessing sex-related differences in CYP activity and related pharmacokinetic parameters, focusing on the impact on anti-infective disposition.

Most studies demonstrate increased CYP3A4 activity in women versus men, although data are conflicting and depend on the enzyme substrate used to assess activity. For example, erythromycin, a CYP3A4 substrate, is cleared more rapidly after intravenous dosing in women than in men (Austin et al. 1980), while metabolism of midazolam, another CYP3A4 substrate, has been shown not to be affected by sex in some studies (Gorski et al. 1998; Kashuba et al. 1998), and the data for the sex-related differences in the metabolism of the CYP3A4 substrate verapamil has been mixed (Kang et al. 2003; Krecic-Shepard et al. 2000a, b). Several factors are likely to contribute to these mixed results, including the variable impact of intestinal versus hepatic enzyme induction, the impact of sex on pharmacokinetic parameters impacting clearance via mechanisms other than drug metabolism, and the extent to which a drug is also a substrate of the efflux transporter p-glycoprotein which may concomitantly impact its pharmacokinetics (Cummins et al. 2002) (See Sect. 4.3.3). Finally, sex-related differences exist in the extent of intestinal and hepatic CYP3A induction by some drugs, such as rifampin. In one study, the extent of induction of oral clearance of oral midazolam by rifampin was greater in men than in women, while the extent of induction of systemic clearance was greater in

women than in men, suggesting differential effect depending on hepatic versus intestinal sites of metabolism (Gorski et al. 2003).

Sex-related differences have also been observed in the activities of other CYP450 enzymes. CYP1A2 and CYP2E1 activities, measured by caffeine and chlorzoxazone metabolism, respectively, were higher in men than in women (Kim and O'Shea 1995; Lucas et al. 1995; Ou-Yang et al. 2000; Relling et al. 1992). In contrast, in Swedish populations, CYP2D6 activity, measured by dextromethorphan metabolism, was higher in women than men (Hagg et al. 2001), while CYP2C19 activity, measured by mephenytoin metabolism, was the same in women not taking contraceptives as in men (Laine et al. 2000).

Phase II Enzymes: The activities of some phase II metabolic enzymes also exhibit sex differences. For instance, studies suggest lower activities of several isoenzymes of the uridine 5-diphosphate glucuronosyltransferase (UGT) superfamily, responsible for the metabolism of the antiretroviral drug raltegravir, in women versus men (Boudikova et al. 1990; Court et al. 2001; Miners et al. 1983; Morissette et al. 2001). Similarly, thiopurine methyltransferase (TPMT) activities were lower in hepatic tissues of women compared with men (Szumlanski et al. 1992), and levels of catechol-O-methyltransferase activity were also lower in women relative to men (Boudikova et al. 1990). Finally, phenol sulfotransferase activity was more than 60 % lower in Finnish women than men (Brittelli et al. 1999).

4.2.5 Elimination

Both drug metabolism (discussed in Sect. 4.2.4) and drug excretion are important for drug elimination. The most common routes of drug excretion include through feces and urine. Renal clearance depends on renal tubular secretion, glomerular filtration, and renal blood flow, all of which are generally higher in men than women (Soldin and Mattison 2009). Differences in glomerular filtration between men and women are generally due to differences in weight. However, the excretion of drugs that exhibit active renal secretion could theoretically be affected by sex-related differences independent of weight, though further research is needed to determine if this mechanism contributes to sex-related differences in the pharmacokinetics of anti-infectives (Gandhi et al. 2004).

4.2.6 Summary

Overall, the clinical data suggest that sex differences in drug disposition are likely to be due to multiple interacting mechanisms impacting drug pharmacokinetics, such as lower body weight, increased gastrointestinal motility, variations in protein binding, altered metabolic enzyme activity, and changes in renal clearance. Despite these reported sex differences, only a few drugs, and even fewer anti-infectives,

have exhibited variable plasma concentrations in men versus women. Furthermore, data on the impact of sex on drug pharmacokinetics are limited. Nonetheless, based on sex-related differences in reported adverse events of several drugs such as antiretrovirals, it remains crucial to understand the potential effects of sex and sex hormones on drug absorption, distribution, metabolism, and elimination, to ensure appropriate drug selection and dosing for men and women. This is particularly important in the setting of drugs requiring long-term use and potential for toxicity.

4.3 Effect of Sex Hormones on Membrane Drug Transporters

4.3.1 Overview of Drug Transporters

For orally administered drugs, hepatic and gastrointestinal transporters influence how much drug escapes first-pass metabolism and enters the body from the gut lumen, controlling access of drug to the systemic circulation (Shugarts and Benet 2009). For drugs that escape first-pass metabolism, renal and hepatic transporters can impact drug clearance. Two major types of transporters, uptake and efflux, exist on intestinal, hepatic, and renal cell membranes and affect pharmacokinetic parameters of drugs that are substrates for these transporters. Uptake transporters generally use chemiosmotic gradients caused by the translocation of ions to move drugs across the membrane. The two major superfamilies of uptake transporters include the SLC and SLCO families. Two members of the SLC family, organic anion transporters (OATs) and organic cation transporters (OCTs), and one member of the SLCO family, organic anion transporting polypeptides (OCTPs), have been well studied. Efflux transporters expressed at these sites are part of the ATP-binding cassette (ABC) superfamily that uses ATP to pump substrates in against an energy source. The major ABC transporters include p-glycoprotein (p-gp), multidrug-resistant proteins (MRPs), and breast cancer-resistant proteins (BCRPs) (Shugarts and Benet 2009; Takano et al. 2006). Expression of drug transporter genes and proteins may vary by sex and thus impact drug disposition differently for women and men.

4.3.2 Effect of Drug Transporters on Pharmacokinetic Parameters

Oral Bioavailability (F): Transporters modulate F by affecting the amount of drug that crosses gut membranes and that is taken up into hepatocytes.

Volume of Distribution (V): Transporters in the gut will not affect V since it relates the amount of drug in the systemic circulation to the amount of drug in the body. However, transporters influence V by mediating transport of drug in and out of many different tissues and organs, such as the brain and liver. For example, for drugs whose site of action is the liver, inhibition of uptake inhibitors results in less drug in the liver and more drug in the plasma, decreasing V/F. In the kidney, inhibition of efflux transporters causes an increase in V (Grover and Benet 2009).

Absorption Half-Life ($t_{1/2,abs}$): Gut transporters can influence K_a and subsequently affect $t_{1/2,\ abs}$. In example, for drugs dependent on gut uptake transporters, inhibition decreases their absorption rate, driving K_a down and increasing $t_{1/2,abs}$. In contrast, induction of gut uptake transporters will decrease $t_{1/2,abs}$.

Area Under the Concentration-Time Curve (AUC): Transporter effects on F will have a corresponding effect on AUC.

Clearance (CL): Transporters on gut membranes affect F exclusively so CL will not change with inhibition or induction of these transporters. For drugs that escape first-pass metabolism, transporters in the liver and kidney can affect clearance of the drug.

For example, both hepatic uptake and efflux transporters expressed on the hepatocyte basolateral membranes affect drug clearance by impacting drug concentrations in hepatocytes (Shugarts and Benet 2009). For drugs metabolized by enzymes within hepatocytes, inhibition of uptake transporters prevents drug metabolism and decreases CL. Similarly, inhibiting uptake transporters decreases CL for drugs eliminated by biliary excretion by preventing drug from entering bile via hepatocytes. In contrast, inhibiting hepatocyte efflux transporters will increase CL.

Elimination Half-Life ($t_{1/2}$): Elimination half-life will increase when transporters predominantly cause an increase in V and will decrease when transporters cause an increase in CL.

4.3.3 Impact of Sex on Drug Transporter Gene and Protein Expression

One proposed mechanism for sex-associated differences in antimicrobial pharmacology is sex-related variation in expression and activity of drug transporter genes and proteins (Ofotokun et al. 2007b). This section will focus on reviewing the current knowledge of this mechanism. Table 4.4 summarizes our current knowledge on known effects of sex or sex hormones on drug transporters and the impact on anti-infectives.

Table 4.4 Effect of sex or sex hormones on transporter expression

Type	Site	Effect of sex/sex hormones	Population studied	Effect of sex on pharmacokinetics	Anti-infective substrates or inhibitors	References
Efflux transporters						
P-glycoprotein	Liver PBMCs Intestine Placental cells CLL cells	M > F Inhibited by progestins M > F Induced by progesterone and β-estradiol M > F	Humans Humans Rats Humans Humans	Not studied Not studied Ivermectin accumulation in gut sac of M > F in presence of p-gp inhibitor 15–40 % decrease in Saquinavir uptake Not studied	*Substrates*: amprenavir, atazanavir, azithromycin, clarithromycin, chloraquine, darunavir, dolutegravir, erythromycin, indinavir, ivermectin, levofloxacin, maraviroc, nelfinavir, ritonavir, quinidine, quinine, saquinavir *Inhibitors*: clarithromycin, cobicistat, erythromycin, etravirine, fluconazole, indinavir, miconazole, nelfinavir, ritonavir, saquinavir	Takano et al. (2006), Steiner et al. (1998), Schuetz et al. (1995), Lifschitz et al. (2006), Frohlich et al. (2004), Coles et al. (2009)
MRP2	Liver	F > M	Rats	Not studied	*Substrates*: azithromycin cephalosporins indinavir ritonavir saquinavir *Inhibitors*: efavirenz nevirapine emtricitabine lamivudine tenofovir	Weiss et al. (2007), Takano et al. (2006), Ruiz et al. (2013), Grandvuinet et al. (2012)

(continued)

Table 4.4 (continued)

Type	Site	Effect of sex/sex hormones	Population studied	Effect of sex on pharmacokinetics	Anti-infective substrates or inhibitors	References
MRP3	Liver Kidney	F > M F > M	Rats	Not studied	*Inhibitors*: efavirenz, emtricitabine, lamivudine, nevirapine, tenofovir	Yacovino and Aleksunes (2012), Weiss et al. (2007), Ruiz et al. (2013)
MRP4	Kidney	F > M	Rats	Not studied	Unknown	Yacovino and Aleksunes (2012)
BCRP	Liver Kidney	M > F M > F	Mice Rats, mice	AUC[g] of nitrofurantoin in females twofold higher than males Not studied	*Substrates*: dolutegravir, lamivudine, nitrofurantoin, zidovudine *Inhibitors*: cobicistat, ritonavir, saquinavir	Merino et al. (2005), Tanaka et al. (2005)
Uptake transporters						
OAT1	Kidney	M > F	Mice, rats	Not studied	*Substrates*: acyclovir, adefovir, cidofovir, ganciclovir tenofovir, zidovudine *Inhibitors*: penicillin G, cephalosporins tetracyclines, cobicistat	Yacovino and Aleksunes (2012), Burckhardt (2012)
OAT2	Kidney	F > M	Mice, rats	Not studied	*Substrates*: acyclovir, erythromycin ganciclovir, penciclovir, tetracyclines *Inhibitors*: chloramphenicol, doxycycline, minocycline	Yacovino and Aleksunes (2012), Burckhardt (2012)
OAT3	Liver	M > F	Mice	Not studied	*Substrates*: acyclovir, cephalosporins, penicillin G, zidovudine *Inhibitors*: ciprofloxacin, zidovudine	Burckhardt (2012), Buist et al. (2003)

OAT5	Kidney	F > M	Mice, rats	Not studied	*Inhibitor*: penicillin G	Yacovino and Aleksunes (2012), Burckhardt (2012)
OCT2	Kidney	M > F	Mice, rats	Not studied	*Substrates*: levofloxacin cephalexin tetracycline trimethoprim saquinavir lopinavir indinavir nelfinavir abacavir emtricitabine lamivudine tenofovir	Yacovino and Aleksunes (2012), Nies et al. (2011)
OATP1A2	Kidney Liver	M > F M > F	Mice, rats	Not studied	*Substrates*: ciprofloxacin, erythromycin, levofloxacin, norfloxacin, darunavir, lopinavir, saquinavir *Inhibitors*: ciprofloxacin, indinavir, moxifloxacin nelfinavir, quinidine, ritonavir, saquinavir	Yacovino and Aleksunes (2012), Roth et al. (2012), Grandvuinet et al. (2012), Rost et al. (2005)
OATP4C1	Kidney	M > F	Mice	Not studied	Unknown	Yacovino and Aleksunes (2012), Roth et al. (2012)
OATP3A1	Kidney	M > F	Mice	Not studied	*Substrates*: penicillin G	Yacovino and Aleksunes (2012), Roth et al. (2012)

M > F greater transporter expression in males than females, *PBMCs* peripheral blood mononuclear cells, *CLL* chronic lymphocytic leukemia, *MRP* multidrug-resistant protein, *F > M* greater transporter expression in females than males, *BCRP* breast cancer-resistant protein, *AUC* area under the concentration-time curve, *OAT* organic anion transporter, *OCT* organic cation transporter, *OATP* organic anion transporting polypeptide

4.3.3.1 ABC Family

P-Glycoprotein (p-gp): P-Glycoprotein (p-gp) is an efflux membrane transporter encoded by the MDR1 gene. Consistent with its role in facilitating excretion of drug across the mucosa of the GI tract and into urine and bile, p-gp is found on the mucosal surfaces of the lower GI tract, the brush borders of proximal tubule renal cells, and the biliary face of hepatocytes (Ambudkar et al. 1999), as well as other sites such as brain and peripheral blood mononuclear cells. P-gp's effect on oral absorption of antimicrobials is best characterized among HIV PIs. For example, in an animal study using a mice model, p-gp was found to limit oral bioavailability of the PIs nelfinavir, saquinavir, and indinavir (Kim et al. 1998).

P-Glycoprotein is the membrane transporter best characterized in the literature regarding sex differences in expression. In a cross-sectional study examining 41 human liver tissue samples, Schuetz et al. found that the hepatic p-gp expression was twofold lower in women compared to men (Schuetz et al. 1995). Similarly, in a cross-sectional study of 61 patients examining chronic lymphocytic leukemia (CLL) cells, 89 % (32/36) of men and only 48 % (12/25) of women were found to be positive for MDR1 expression (Steiner et al. 1998). In an animal study using rats, gut sac accumulation of the antiparasitic drug ivermectin, a substrate for p-glycoprotein, was measured in male and female rats in the presence of a p-gp inhibitor; increased ivermectin accumulation was noted in both groups but was higher in males than females, consistent with lower intestinal expression of p-gp in females (Lifschitz et al. 2006).

To our knowledge, there are no in vivo human data investigating the role of sex hormones in the modulation of p-gp. However, p-gp inhibition by progestins was seen in vitro using two p-gp overexpressing cell lines and ex vivo in human peripheral blood mononuclear cells (Frohlich et al. 2004). In contrast, an in vitro study found that both 17β-estradiol and progesterone caused induction of MDR1 RNA and subsequent p-gp function in both p-gp overexpressing cell lines and placental cells, resulting in a 15–40 % decrease in saquinavir uptake (Coles et al. 2009). Concentrations of progesterone in this in vitro study were comparable to levels frequently attained by women during pregnancy, suggesting that these changes may be clinically significant.

Multidrug-Resistant Protein Transporters (MRPs): Animal studies of MRP transporter expression on liver and kidney have revealed differences mediated by sex steroid hormones. For example, in rat livers, the presence of ethynylestradiol resulted in upregulation of MRP3 expression and downregulation of MRP2 expression (Ruiz et al. 2013). In rat kidneys, estradiol stimulated MRP3 expression and testosterone suppressed MRP3 and MRP4 expression (Yacovino and Aleksunes 2012); not surprisingly, pregnant mice showed upregulation of MRP3 and downregulation of MRP2 and MRP4 expression in their kidneys (Yacovino et al. 2013). While cephalosporin antibiotics and several HIV PIs are substrates for MRP class of transporters, to our knowledge there are no studies examining the

effect of sex hormones on antibiotic pharmacokinetics or drug efficacy, and transporter expression may not correlate to different species.

Breast Cancer-Resistant Protein (BCRP) Transporters: Expression of the BCRP transporter was found to be higher in male mice livers compared to females; after administration of the antibiotic nitrofurantoin, the drug AUC was twofold higher in female than male mice (Merino et al. 2005). Expression of the BCRP transporter gene has also been found to be higher in the kidneys of mice; however, to our knowledge, there are no studies examining the effect of this on antimicrobial pharmacokinetics (Tanaka et al. 2005).

4.3.3.2 SLC and SLCO Families

Organic Anion Transporters (OATs) and Organic Cation Transporters (OCTs): Hepatic OAT3 expression in mice was found to be higher in males than females (Buist et al. 2003). Additionally, sex steroid hormones have been shown to affect OAT expression in kidneys of rats and mice. OAT1 is the rodent male-predominant transporter, while OAT2 and OAT5 are female predominant (Yacovino and Aleksunes 2012). Kidneys of castrated males were found to have upregulation of mRNA and protein expression of OAT2 and OAT5, and this expression was further enhanced with the administration of estradiol. In addition, sex differences have been found in OCT2 transporter expression rat kidneys, with higher expression in male than female rats (Urakami et al. 1999).

Organic Anion Transporting Polypeptides: Expression of rat liver OATP1A4 (human protein OATP1A2) was found to be higher in male than female mice (Rost et al. 2005). Similarly, rat and mouse kidney OATP1A1 (also human protein OATP1A2) and mouse kidney OATP3A1 and OATP4C1 were found to be higher in males than female (Yacovino and Aleksunes 2012).

For the SLC and SLCO families of transporters, evidence suggests that these transporters, particularly those located on the kidneys, may play a pharmacological role in the clearance of exogenous cations from the blood. However, these data are limited to rodents and may not be applicable to other species, including humans. Furthermore, it remains unknown whether the discrepancy in uptake transporter expression between the sexes would result in clinically significant differences in clearance of antimicrobials.

4.3.4 Summary

While clinical data suggest that sex steroid hormones can influence expression of uptake and efflux transporters, the evidence predominantly involves rodent studies and often lacks comparisons of drug pharmacokinetic measures or other clinically significant outcomes. Thus, with most of these data, it is unclear whether these discrepancies in transporter expression among the sexes would result in clinically

relevant differences in drug pharmacokinetics or pharmacodynamics. Nonetheless, it is important to understand the potential effects that sex steroid hormones can have on drug transporters, particularly in vulnerable populations such as pregnant HIV-infected women where attaining adequate concentrations of antiretroviral medications is crucial for virologic suppression and the health of both mother and fetus.

4.4 Special Considerations

4.4.1 Pregnancy

Pregnancy results in multiple physiologic changes that affect plasma drug concentrations and drug pharmacokinetics. Regarding drug absorption, pregnancy is associated with prolonged gastrointestinal transit time (Singer and Brandt 1991). Drug distribution is also affected due to increases in plasma volume and total body water resulting in increased volume of distribution, increased cardiac output and changes in regional blood flow, compensated respiratory alkalosis, and decreases in plasma albumin (Haram et al. 1983) and possibly other binding proteins (Aquirre et al. 1988; Chu et al. 1981; Haram et al. 1983; Hill and Abramson 1988; Notarianni 1990; Perucca and Crema 1982; Wood and Wood 1981). Thus, drugs that are highly protein bound may demonstrate higher free levels (and therefore higher activity) due to decreased protein-binding availability during pregnancy (Costantine 2014). Drug metabolism is altered during pregnancy due to increased hepatic blood flow and hormonal effects on hepatic metabolic and transporter enzymes, and the placental and fetal tissues may also contribute to drug metabolism. Finally, these metabolic changes as well as increased renal blood flow, increased glomerular filtration rate, and elimination of drugs by the fetus may contribute to increased drug elimination during pregnancy.

These changes may affect requirements for drug dosing and potentially modify a pregnant woman's susceptibility to drug toxicity. For example, plasma concentrations of the HIV PIs ritonavir-boosted lopinavir, atazanavir, darunavir, and nelfinavir are reduced during the second and/or third trimesters (Rakhmanina et al. 2012; Roustit et al. 2008). Thus, HIV PIs without ritonavir boosting are not recommended during pregnancy, and doses of ritonavir-boosted PIs may need to be increased during the second and/or third trimesters, particularly in the setting of concomitant medications that result in reduced plasma drug concentrations (Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission 2014). Finally, treatment considerations for infectious diseases during pregnancy should include avoidance of anti-infectives with potential teratogenicity whenever possible. Details on treatment of infections during pregnancy will be covered in subsequent chapters.

4.4.2 Menopause

Menopause is associated with a cessation of estradiol and progesterone production by the ovaries and an overt decline in circulating levels of these hormones and signaling in target tissues. Most studies assessing the effect of menopause on drug disposition have evaluated its impact on drug metabolism and demonstrate conflicting data on whether the hormonal changes associated with menopause result in alterations in hepatic drug metabolism. Data are conflicting regarding whether the hormonal changes associated with menopause result in pharmacokinetic changes. Studies of the CYP3A4 substrates midazolam (a sedative) and erythromycin (an antibacterial drug) demonstrated no differences in drug metabolism among women by menopausal status or hormone replacement therapy (Gorski et al. 2000; Harris et al. 1996), although one study demonstrated lower midazolam clearance in postmenopausal women that was not reversed by hormone replacement therapy (Fleishaker et al. 1999). On the other hand, clearance of the CYP3A4 substrate alfentanil was higher in women above 50 years versus younger women, a difference not noted in men (Lemmens et al. 1990).

4.4.3 Contraceptive Hormones

Increased levels of circulating estrogen and/or progesterone noted in women taking contraceptive hormones may also affect protein-binding and hepatic metabolic enzyme activity. Levels and glycosylation of plasma AAG and serum-binding globulins may also be affected by exogenous estrogens, resulting in decreased AAG, increased glycosylation, and increased serum-binding globulins (Wiegratz et al. 2003a). The use of combined oral contraceptives was shown to reduce CYP2C19 activity in two studies, presumably related to the ethinyl estradiol component of the formulation (Hagg et al. 2001; Laine et al. 2000). Finally, paracetamol clearance was higher in women using oral contraceptives compared with women who were not, representing increased glucuronidation (Miners et al. 1983).

Finally, the impact of anti-infectives on the disposition of contraceptive hormones is also of paramount importance, particularly for anti-infectives that could potentially impact contraceptive efficacy. Despite this concern, the concomitant administration of most antibiotics and antifungals has not been shown to reduce plasma levels of contraceptive hormones (Archer and Archer 2002; Dickinson et al. 2001; Centers for Disease Control and Prevention (CDC) 2010). However, the antimycobacterial drugs rifampin and, to a lesser degree, rifabutin induce hepatic CYP450, thereby impacting hormone concentrations resulting from both combined hormonal contraceptives and progestin-only pills and implants. Some antiretroviral drugs may also affect the level of steroid hormones in the blood due to inhibition or induction of CYP450 pathways, potentially resulting in increased side

effects or decreased contraceptive efficacy. In particular, ritonavir-boosted HIV PIs may decrease steroid hormone disposition in combined oral contraceptives, which may lead to contraceptive failure (German et al. 2011; Carten et al. 2010). The pharmacologic booster cobicistat may result in increased progestin levels through CYP450 inhibition. Additionally, the NNRTI efavirenz can decrease in circulating progestins through inhibition of metabolism (Leticee et al. 2012; Vogler et al. 2010). The degree of reduction in method effectiveness with efavirenz use may vary based on the level of circulating progestin resulting from the contraceptive hormone. For instance, the use of efavirenz has also been associated with increased risk of failure of progestin implants, which generally result in lower levels of circulating progestins (Leticee et al. 2012).

4.5 Conclusions and Future Directions

Existing evidence suggests that women and men differ in their rates of disposition of certain drugs; however, these sex-related differences are poorly characterized for the majority of anti-infectives. Sex- and sex steroid-mediated effects influence every phase of the drug disposition process including absorption, distribution, metabolism, and excretion and alter to varying degrees the activities of drug-metabolizing and transporter systems. The therapeutic implication of sex-related differences in drugs for which data exist appear to be marginal, but many of these studies are limited by the small number of women participants or the use of only animal models. Comprehensive evaluation of the sex-related effect on anti-infective drug activity is further limited by stringent eligibility criteria imposed during early phase clinical studies that limit participation of women, particularly pregnant women and those of childbearing age.

To enhance the effectiveness and reduce toxicities of anti-infective drugs among women, further studies are needed to define the role of sex on the pharmacokinetics and pharmacodynamics of commonly used anti-infectives. These studies should elucidate the mechanisms underlying any observed differences, particularly for those drugs with narrow therapeutic windows as well as those that require long-term use. To accomplish this, it is imperative that future clinical studies prioritize the recruitment of women representing the entire spectrum from their reproductive years through menopause and beyond.

References

Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM (1999) Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol 39:361–398. doi:10.1146/annurev.pharmtox.39.1.361

Anderson PL, Kakuda TN, Kawle S, Fletcher CV (2003) Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in HIV-infected individuals. AIDS 17(15):2159–2168. doi:10.1097/01.aids.0000076338.42412.62

Aquirre C, Rodriguez-Sasiain JM, Navajas P, Calvo R (1988) Plasma protein binding of penbutolol in pregnancy. Eur J Drug Metab Pharmacokinet 13(1):23–26

Archer JS, Archer DF (2002) Oral contraceptive efficacy and antibiotic interaction: a myth debunked. J Am Acad Dermatol 46(6):917–923

Austin KL, Mather LE, Philpot CR, McDonald PJ (1980) Intersubject and dose-related variability after intravenous administration of erythromycin. Br J Clin Pharmacol 10(3):273–279

Bertino JS Jr, Nafziger AN (1996) Pharmacokinetics of oral fleroxacin in male and premenopausal female volunteers. Antimicrob Agents Chemother 40(3):789–791

Boudikova B, Szumlanski C, Maidak B, Weinshilboum R (1990) Human liver catechol-O-methyltransferase pharmacogenetics. Clin Pharmacol Ther 48(4):381–389

Brittelli A, De Santi C, Raunio H, Pelkonen O, Rossi G, Pacifici GM (1999) Interethnic and interindividual variabilities of platelet sulfotransferases activity in Italians and Finns. Eur J Clin Pharmacol 55(9):691–695

Buist SC, Cherrington NJ, Klaassen CD (2003) Endocrine regulation of rat organic anion transporters. Drug Metab Dispos 31(5):559–564

Burckhardt G (2012) Drug transport by organic anion transporters (OATs). Pharmacol Ther 136(1):106–130. doi:10.1016/j.pharmthera.2012.07.010

Carcas AJ, Guerra P, Frias J, Soto A, Fernandez-Aijon A, Montuenga C, Govantes C (2001) Gender differences in the disposition of metronidazole. Int J Clin Pharmacol Ther 39(5): 213–218

Carten M, Kiser J, Kwara A, MaWhinney S, Cu-Uvin S (2010) Pharmacokinetic (PK) interactions between the hormonal emergency contraception Plan B (levonorgestrel) and efavirenz (EFV). In: 17th conference on retroviruses and opportunistic infection, San Francisco, CA, Feb 2010

Centers for Disease Control and Prevention (CDC) (2010) U.S. medical eligibility criteria for contraceptive use. Morbidity and Mortality Weekly Report (MMWR)

Chen TS, Doong ML, Chang FY, Lee SD, Wang PS (1995) Effects of sex steroid hormones on gastric emptying and gastrointestinal transit in rats. Am J Physiol 268(1 Pt 1):G171–G176

Chen ML, Lee SC, Ng MJ, Schuirmann DJ, Lesko LJ, Williams RL (2000) Pharmacokinetic analysis of bioequivalence trials: implications for sex-related issues in clinical pharmacology and biopharmaceutics. Clin Pharmacol Ther 68(5):510–521. doi:10.1067/mcp.2000.111184

Chu CY, Singla VP, Wang HP, Sweet B, Lai LT (1981) Plasma alpha 1-acid glycoprotein levels in pregnancy. Clin Chim Acta 112(2):235–240

Coles LD, Lee IJ, Voulalas PJ, Eddington ND (2009) Estradiol and progesterone-mediated regulation of P-gp in P-gp overexpressing cells (NCI-ADR-RES) and placental cells (JAR). Mol Pharm 6(6):1816–1825. doi:10.1021/mp900077q

Collen MJ, Abdulian JD, Chen YK (1994) Age does not affect basal gastric acid secretion in normal subjects or in patients with acid-peptic disease. Am J Gastroenterol 89(5):712–716

Coskun T, Sevinc A, Tevetoglu I, Alican I, Kurtel H, Yegen BC (1995) Delayed gastric emptying in conscious male rats following chronic estrogen and progesterone treatment. Res Exp Med 195(1):49–54

Costantine MM (2014) Physiologic and pharmacokinetic changes in pregnancy. Front Pharmacol 5:65. doi:10.3389/fphar.2014.00065

Court MH, Duan SX, von Moltke LL, Greenblatt DJ, Patten CJ, Miners JO, Mackenzie PI (2001) Interindividual variability in acetaminophen glucuronidation by human liver microsomes: identification of relevant acetaminophen UDP-glucuronosyltransferase isoforms. J Pharmacol Exp Ther 299(3):998–1006

Cummins CL, Wu CY, Benet LZ (2002) Sex-related differences in the clearance of cytochrome P450 3A4 substrates may be caused by P-glycoprotein. Clin Pharmacol Ther 72(5):474–489. doi:10.1067/mcp.2002.128388

Degen LP, Phillips SF (1996) Variability of gastrointestinal transit in healthy women and men. Gut 39(2):299–305

del Carmen Carrasco-Portugal M, Lujan M, Flores-Murrieta FJ (2008) Evaluation of gender in the oral pharmacokinetics of clindamycin in humans. Biopharm Drug Dispos 29(7):427–430. doi:10.1002/bdd.624

Delille CA, Pruett ST, Marconi VC, Lennox JL, Armstrong WS, Arrendale RF, Sheth AN, Easley KA, Acosta EP, Vunnava A, Ofotokun I (2014) Effect of protein binding on unbound atazanavir and darunavir cerebrospinal fluid concentrations. J Clin Pharmacol 54:1063–1071. doi:10.1002/jcph.298

Dickinson BD, Altman RD, Nielsen NH, Sterling ML (2001) Drug interactions between oral contraceptives and antibiotics. Obstet Gynecol 98(5 Pt 1):853–860

Fleishaker JC, Pearson LK, Pearson PG, Wienkers LC, Hopkins NK, Peters GR (1999) Hormonal effects on tirilazad clearance in women: assessment of the role of CYP3A. J Clin Pharmacol 39(3):260–267

Fletcher CV, Jiang H, Brundage RC, Acosta EP, Haubrich R, Katzenstein D, Gulick RM (2004) Sex-based differences in saquinavir pharmacology and virologic response in AIDS Clinical Trials Group Study 359. J Infect Dis 189(7):1176–1184. doi:10.1086/382754

Frohlich M, Albermann N, Sauer A, Walter-Sack I, Haefeli WE, Weiss J (2004) In vitro and ex vivo evidence for modulation of P-glycoprotein activity by progestins. Biochem Pharmacol 68(12):2409–2416. doi:10.1016/j.bcp.2004.08.026

Gandhi M, Aweeka F, Greenblatt RM, Blaschke TF (2004) Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol 44:499–523. doi:10.1146/annurev.pharmtox.44.101802.121453

German P, Wang M, Warren D, Kearney B (2011) Pharmacokinetic interaction between Norgestimate/Ethinyl Estradiol and EVG/COBI/FTC/TDF single tablet regimen. In: 12th international workshop on clinical pharmacology of HIV therapy, Miami, FL, Apr 2011

Gorski JC, Jones DR, Haehner-Daniels BD, Hamman MA, O'Mara EM Jr, Hall SD (1998) The contribution of intestinal and hepatic CYP3A to the interaction between midazolam and clarithromycin. Clin Pharmacol Ther 64(2):133–143. doi:10.1016/s0009-9236(98)90146-1

Gorski JC, Wang Z, Haehner-Daniels BD, Wrighton SA, Hall SD (2000) The effect of hormone replacement therapy on CYP3A activity. Clin Pharmacol Ther 68(4):412–417. doi:10.1067/mcp.2000.110560

Gorski JC, Vannaprasaht S, Hamman MA, Ambrosius WT, Bruce MA, Haehner-Daniels B, Hall SD (2003) The effect of age, sex, and rifampin administration on intestinal and hepatic cytochrome P450 3A activity. Clin Pharmacol Ther 74(3):275–287. doi:10.1016/s0009-9236(03)00187-5

Grandvuinet AS, Vestergaard HT, Rapin N, Steffansen B (2012) Intestinal transporters for endogenic and pharmaceutical organic anions: the challenges of deriving in-vitro kinetic parameters for the prediction of clinically relevant drug-drug interactions. J Pharm Pharmacol 64(11):1523–1548. doi:10.1111/j.2042-7158.2012.01505.x

Grover A, Benet LZ (2009) Effects of drug transporters on volume of distribution. AAPS J 11(2):250–261. doi:10.1208/s12248-009-9102-7

Guo T, Sun WJ, Xia DY, Zhao LS (2010) The pharmacokinetics of fluconazole in healthy Chinese adult volunteers: influence of ethnicity and gender. J Clin Pharm Ther 35(2):231–237. doi:10.1111/j.1365-2710.2009.01097.x

Hagg S, Spigset O, Dahlqvist R (2001) Influence of gender and oral contraceptives on CYP2D6 and CYP2C19 activity in healthy volunteers. Br J Clin Pharmacol 51(2):169–173

Haram K, Augensen K, Elsayed S (1983) Serum protein pattern in normal pregnancy with special reference to acute-phase reactants. Br J Obstet Gynaecol 90(2):139–145

Harris RZ, Tsunoda SM, Mroczkowski P, Wong H, Benet LZ (1996) The effects of menopause and hormone replacement therapies on prednisolone and erythromycin pharmacokinetics. Clin Pharmacol Ther 59(4):429–435. doi:10.1016/S0009-9236(96)90112-5

Hill MD, Abramson FP (1988) The significance of plasma protein binding on the fetal/maternal distribution of drugs at steady-state. Clin Pharmacokinet 14(3):156–170. doi:10.2165/00003088-198814030-00004

Hreiche R, Morissette P, Turgeon J (2008) Drug-induced long QT syndrome in women: review of current evidence and remaining gaps. Gend Med 5(2):124–135. doi:10.1016/j.genm.2008.05.005

Hutson WR, Roehrkasse RL, Wald A (1989) Influence of gender and menopause on gastric emptying and motility. Gastroenterology 96(1):11–17

Iwainsky H, Winsel K, Werner E, Eule H (1976) On the pharmacokinetics of rifampicin during treatment with intermittent administration. II. Influence of age and sex and of the patients. Scand J Respir Dis 57(1):5–11

Kamm MA, Farthing MJ, Lennard-Jones JE (1989) Bowel function and transit rate during the menstrual cycle. Gut 30(5):605–608

Kang D, Verotta D, Krecic-Shepard ME, Modi NB, Gupta SK, Schwartz JB (2003) Population analyses of sustained-release verapamil in patients: effects of sex, race, and smoking. Clin Pharmacol Ther 73(1):31–40. doi:10.1067/mcp.2003.21

Kashuba AD, Nafziger AN (1998) Physiological changes during the menstrual cycle and their effects on the pharmacokinetics and pharmacodynamics of drugs. Clin Pharmacokinet 34(3):203–218. doi:10.2165/00003088-199834030-00003

Kashuba AD, Bertino JS Jr, Rocci ML Jr, Kulawy RW, Beck DJ, Nafziger AN (1998) Quantification of 3-month intraindividual variability and the influence of sex and menstrual cycle phase on CYP3A activity as measured by phenotyping with intravenous midazolam. Clin Pharmacol Ther 64(3):269–277. doi:10.1016/s0009-9236(98)90175-8

Kim RB, O'Shea D (1995) Interindividual variability of chlorzoxazone 6-hydroxylation in men and women and its relationship to CYP2E1 genetic polymorphisms. Clin Pharmacol Ther 57(6):645–655. doi:10.1016/0009-9236(95)90227-9

Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Roden DM, Wilkinson GR (1998) The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. J Clin Invest 101(2):289–294. doi:10.1172/JCI1269

Kishino S, Nomura A, Itoh S, Nakagawa T, Takekuma Y, Sugawara M, Furukawa H, Todo S, Miyazaki K (2002) Age- and gender-related differences in carbohydrate concentrations of alpha1-acid glycoprotein variants and the effects of glycoforms on their drug-binding capacities. Eur J Clin Pharmacol 58(9):621–628. doi:10.1007/s00228-002-0530-x

Knight V, Yu CP, Gilbert BE, Divine GW (1988) Estimating the dosage of ribavirin aerosol according to age and other variables. J Infect Dis 158(2):443–448

Krecic-Shepard ME, Barnas CR, Slimko J, Jones MP, Schwartz JB (2000a) Gender-specific effects on verapamil pharmacokinetics and pharmacodynamics in humans. J Clin Pharmacol 40(3):219–230

Krecic-Shepard ME, Barnas CR, Slimko J, Schwartz JB (2000b) Faster clearance of sustained release verapamil in men versus women: continuing observations on sex-specific differences after oral administration of verapamil. Clin Pharmacol Ther 68(3):286–292. doi:10.1067/mcp.2000.109356

Laine K, Tybring G, Bertilsson L (2000) No sex-related differences but significant inhibition by oral contraceptives of CYP2C19 activity as measured by the probe drugs mephenytoin and omeprazole in healthy Swedish white subjects. Clin Pharmacol Ther 68(2):151–159. doi:10.1067/mcp.2000.108949

Lemmens HJ, Burm AG, Hennis PJ, Gladines MP, Bovill JG (1990) Influence of age on the pharmacokinetics of alfentanil. Gender dependence. Clin Pharmacokinet 19(5):416–422. doi:10.2165/00003088-199019050-00005

Leticee N, Viard JP, Yamgnane A, Karmochkine M, Benachi A (2012) Contraceptive failure of etonogestrel implant in patients treated with antiretrovirals including efavirenz. Contraception 85(4):425–427. doi:10.1016/j.contraception.2011.09.005

Lifschitz A, Ballent M, Virkel G, Sallovitz J, Lanusse C (2006) Sex-related differences in the gastrointestinal disposition of ivermectin in the rat: P-glycoprotein involvement and itraconazole modulation. J Pharm Pharmacol 58(8):1055–1062. doi:10.1211/jpp.58.8.0005

Liu CY, Chen LB, Liu PY, Xie DP, Wang PS (2002) Effects of progesterone on gastric emptying and intestinal transit in male rats. World J Gastroenterol 8(2):338–341

Lucas D, Menez C, Girre C, Berthou F, Bodenez P, Joannet I, Hispard E, Bardou LG, Menez JF (1995) Cytochrome P450 2E1 genotype and chlorzoxazone metabolism in healthy and alcoholic Caucasian subjects. Pharmacogenetics 5(5):298–304

Mazhude C, Jones S, Murad S, Taylor C, Easterbrook P (2002) Female sex but not ethnicity is a strong predictor of non-nucleoside reverse transcriptase inhibitor-induced rash. AIDS 16(11): 1566–1568

Merino G, van Herwaarden AE, Wagenaar E, Jonker JW, Schinkel AH (2005) Sex-dependent expression and activity of the ATP-binding cassette transporter breast cancer resistance protein (BCRP/ABCG2) in liver. Mol Pharmacol 67(5):1765–1771. doi:10.1124/mol.105.011080

Miners JO, Attwood J, Birkett DJ (1983) Influence of sex and oral contraceptive steroids on paracetamol metabolism. Br J Clin Pharmacol 16(5):503–509

Moore AL, Mocroft A, Madge S, Devereux H, Wilson D, Phillips AN, Johnson M (2001) Gender differences in virologic response to treatment in an HIV-positive population: a cohort study. J Acquir Immune Defic Syndr 26(2):159–163

Morissette P, Albert C, Busque S, St-Louis G, Vinet B (2001) In vivo higher glucuronidation of mycophenolic acid in male than in female recipients of a cadaveric kidney allograft and under immunosuppressive therapy with mycophenolate mofetil. Ther Drug Monit 23(5):520–525

Nicolson TJ, Mellor HR, Roberts RR (2010) Gender differences in drug toxicity. Trends Pharmacol Sci 31(3):108–114. doi:10.1016/j.tips.2009.12.001

Nies AT, Koepsell H, Damme K, Schwab M (2011) Organic cation transporters (OCTs, MATEs), in vitro and in vivo evidence for the importance in drug therapy. Handb Exp Pharmacol 201: 105–167. doi:10.1007/978-3-642-14541-4_3

Notarianni LJ (1990) Plasma protein binding of drugs in pregnancy and in neonates. Clin Pharmacokinet 18(1):20–36

Ofotokun I (2005) Sex differences in the pharmacologic effects of antiretroviral drugs: potential roles of drug transporters and phase 1 and 2 metabolizing enzymes. Top HIV Med 13(2):79–83

Ofotokun I, Chuck SK, Binongo JN, Palau M, Lennox JL, Acosta EP (2007a) Lopinavir/Ritonavir pharmacokinetic profile: impact of sex and other covariates following a change from twice-daily to once-daily therapy. J Clin Pharmacol 47(8):970–977. doi:10.1177/0091270007302564

Ofotokun I, Chuck SK, Hitti JE (2007b) Antiretroviral pharmacokinetic profile: a review of sex differences. Gend Med 4(2):106–119

Ofotokun I, Lennox JL, Eaton ME, Ritchie JC, Easley KA, Masalovich SE, Long MC, Acosta EP (2011) Immune activation mediated change in alpha-1-acid glycoprotein: impact on total and free lopinavir plasma exposure. J Clin Pharmacol 51(11):1539–1548. doi:10.1177/0091270010385118

Ou-Yang DS, Huang SL, Wang W, Xie HG, Xu ZH, Shu Y, Zhou HH (2000) Phenotypic polymorphism and gender-related differences of CYP1A2 activity in a Chinese population. Br J Clin Pharmacol 49(2):145–151

Pai MP, Schriever CA, Diaz-Linares M, Novak RM, Rodvold KA (2004) Sex-related differences in the pharmacokinetics of once-daily saquinavir soft-gelatin capsules boosted with low-dose ritonavir in patients infected with human immunodeficiency virus type 1. Pharmacotherapy 24(5):592–599

Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission (2014) Recommendations for use of antiretroviral drugs in pregnant HIV-1-infected women for maternal health and interventions to reduce perinatal HIV transmission in the United States. http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf. Accessed 6 May 2014

Pernerstorfer-Schoen H, Jilma B, Perschler A, Wichlas S, Schindler K, Schindl A, Rieger A, Wagner OF, Quehenberger P (2001) Sex differences in HAART-associated dyslipidaemia. AIDS 15(6):725–734

Perucca E, Crema A (1982) Plasma protein binding of drugs in pregnancy. Clin Pharmacokinet 7(4):336–352

Rakhmanina NY, Dirajlal-Fargo S, Capparelli EV, Mirochnik M (2012) Pharmacokinetic considerations of perinatal antiretroviral therapy. Curr Drug Metab 13(6):744–759

Relling MV, Lin JS, Ayers GD, Evans WE (1992) Racial and gender differences in N-acetyltransferase, xanthine oxidase, and CYP1A2 activities. Clin Pharmacol Ther 52(6): 643–658

Rodriguez I, Kilborn MJ, Liu XK, Pezzullo JC, Woosley RL (2001) Drug-induced QT prolongation in women during the menstrual cycle. JAMA 285(10):1322–1326

Rost D, Kopplow K, Gehrke S, Mueller S, Friess H, Ittrich C, Mayer D, Stiehl A (2005) Gender-specific expression of liver organic anion transporters in rat. Eur J Clin Investig 35(10): 635–643. doi:10.1111/j.1365-2362.2005.01556.x

Roth M, Obaidat A, Hagenbuch B (2012) OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. Br J Pharmacol 165(5):1260–1287. doi:10.1111/j.1476-5381.2011.01724.x

Roustit M, Jlaiel M, Leclercq P, Stanke-Labesque F (2008) Pharmacokinetics and therapeutic drug monitoring of antiretrovirals in pregnant women. Br J Clin Pharmacol 66(2):179–195. doi:10.1111/j.1365-2125.2008.03220.x

Ruiz ML, Rigalli JP, Arias A, Villanueva S, Banchio C, Vore M, Mottino AD, Catania VA (2013) Induction of hepatic multidrug resistance-associated protein 3 by ethynylestradiol is independent of cholestasis and mediated by estrogen receptor. Drug Metab Dispos 41(2):275–280. doi:10.1124/dmd.112.047357

Schuetz EG, Furuya KN, Schuetz JD (1995) Interindividual variation in expression of P-glycoprotein in normal human liver and secondary hepatic neoplasms. J Pharmacol Exp Ther 275(2):1011–1018

Shugarts S, Benet LZ (2009) The role of transporters in the pharmacokinetics of orally administered drugs. Pharm Res 26(9):2039–2054. doi:10.1007/s11095-009-9924-0

Singer AJ, Brandt LJ (1991) Pathophysiology of the gastrointestinal tract during pregnancy. Am J Gastroenterol 86(12):1695–1712

Soldin OP, Mattison DR (2009) Sex differences in pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 48(3):143–157. doi:10.2165/00003088-200948030-00001

Soldin OP, Chung SH, Mattison DR (2011) Sex differences in drug disposition. J Biomed Biotechnol 2011:187103. doi:10.1155/2011/187103

Sowinski KM, Abel SR, Clark WR, Mueller BA (1999) Effect of gender on the pharmacokinetics of ofloxacin. Pharmacotherapy 19(4):442–446

Squires KE, Johnson M, Yang R, Uy J, Sheppard L, Absalon J, McGrath D (2011) Comparative gender analysis of the efficacy and safety of atazanavir/ritonavir and lopinavir/ritonavir at 96 weeks in the CASTLE study. J Antimicrob Chemother 66(2):363–370. doi:10.1093/jac/dkq457

Steiner H, Polliack A, Kimchi-Sarfaty C, Libster D, Fibach E, Rund D (1998) Differences in rhodamine-123 efflux in B-type chronic lymphocytic leukemia suggest possible gender and stage variations in drug-resistance gene activity. Ann Hematol 76(5):189–194

Succari M, Foglietti MJ, Percheron F (1990) Microheterogeneity of alpha 1-acid glycoprotein: variation during the menstrual cycle in healthy women, and profile in women receiving estrogen-progestogen treatment. Clin Chim Acta 187(3):235–241

Szumlanski CL, Honchel R, Scott MC, Weinshilboum RM (1992) Human liver thiopurine methyltransferase pharmacogenetics: biochemical properties, liver-erythrocyte correlation and presence of isozymes. Pharmacogenetics 2(4):148–159

Takano M, Yumoto R, Murakami T (2006) Expression and function of efflux drug transporters in the intestine. Pharmacol Ther 109(1–2):137–161. doi:10.1016/j.pharmthera.2005.06.005

Tanaka Y, Slitt AL, Leazer TM, Maher JM, Klaassen CD (2005) Tissue distribution and hormonal regulation of the breast cancer resistance protein (Bcrp/Abcg2) in rats and mice. Biochem Biophys Res Commun 326(1):181–187. doi:10.1016/j.bbrc.2004.11.012

Urakami Y, Nakamura N, Takahashi K, Okuda M, Saito H, Hashimoto Y, Inui K (1999) Gender differences in expression of organic cation transporter OCT2 in rat kidney. FEBS Lett 461(3): 339–342

Vogler MA, Patterson K, Kamemoto L, Park JG, Watts H, Aweeka F, Klingman KL, Cohn SE (2010) Contraceptive efficacy of oral and transdermal hormones when co-administered with protease inhibitors in HIV-1-infected women: pharmacokinetic results of ACTG trial A5188. J Acquir Immune Defic Syndr 55(4):473–482. doi:10.1097/QAI.0b013e3181eb5ff5

von Hentig N, Babacan E, Lennemann T, Knecht G, Carlebach A, Harder S, Staszewski S, Haberl A (2008) The steady-state pharmacokinetics of atazanavir/ritonavir in HIV-1-infected adult outpatients is not affected by gender-related co-factors. J Antimicrob Chemother 62(3): 579–582. doi:10.1093/jac/dkn204

Walle UK, Fagan TC, Topmiller MJ, Conradi EC, Walle T (1994) The influence of gender and sex steroid hormones on the plasma binding of propranolol enantiomers. Br J Clin Pharmacol 37(1):21–25

Waxman DJ, Holloway MG (2009) Sex differences in the expression of hepatic drug metabolizing enzymes. Mol Pharmacol 76(2):215–228. doi:10.1124/mol.109.056705

Weiss J, Theile D, Ketabi-Kiyanvash N, Lindenmaier H, Haefeli WE (2007) Inhibition of MRP1/ABCC1, MRP2/ABCC2, and MRP3/ABCC3 by nucleoside, nucleotide, and non-nucleoside reverse transcriptase inhibitors. Drug Metab Dispos 35(3):340–344. doi:10.1124/dmd.106.012765

Wiegratz I, Kutschera E, Lee JH, Moore C, Mellinger U, Winkler UH, Kuhl H (2003a) Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. Contraception 67(1):25–32

Wiegratz I, Kutschera E, Lee JH, Moore C, Mellinger U, Winkler UH, Kuhl H (2003b) Effect of four oral contraceptives on thyroid hormones, adrenal and blood pressure parameters. Contraception 67(5):361–366

Wood M, Wood AJ (1981) Changes in plasma drug binding and alpha 1-acid glycoprotein in mother and newborn infant. Clin Pharmacol Ther 29(4):522–526

Wrighton SA, Stevens JC (1992) The human hepatic cytochromes P450 involved in drug metabolism. Crit Rev Toxicol 22(1):1–21. doi:10.3109/10408449209145319

Yacovino LL, Aleksunes LM (2012) Endocrine and metabolic regulation of renal drug transporters. J Biochem Mol Toxicol 26(10):407–421. doi:10.1002/jbt.21435

Yacovino LL, Gibson CJ, Aleksunes LM (2013) Down-regulation of brush border efflux transporter expression in the kidneys of pregnant mice. Drug Metab Dispos 41(2):320–325. doi:10.1124/dmd.112.047092

Zhou XJ, Sheiner LB, D'Aquila RT, Hughes MD, Hirsch MS, Fischl MA, Johnson VA, Myers M, Sommadossi JP (1999) Population pharmacokinetics of nevirapine, zidovudine, and didanosine in human immunodeficiency virus-infected patients. The national institute of allergy and infectious diseases AIDS clinical trials group protocol 241 investigators. Antimicrob Agents Chemother 43(1):121–128

Chapter 5
Sex Differences in the Manifestations of HIV-1 Infection

Morgane Griesbeck and Marcus Altfeld

Abstract Sex differences have been reported for multiple aspects of human immunodeficiency virus (HIV)-1 infection, including transmission, pathogenesis, morbidity, mortality, and responses to antiretroviral treatment. Epidemiological studies on sex differences in HIV-1 infection are numerous and in some instances controversial. The intrinsic interplay between multilayered socioeconomic factors and biological factors renders drawing definitive conclusions on sex differences in HIV-1 infection challenging. Nevertheless, some findings such as the lower viremia observed in women than in men have been consistently observed in multiple studies. It is also generally acknowledged that women display a greater susceptibility to HIV-1 acquisition. The simian immunodeficiency virus (SIV) model has been critical for understanding the biological characteristics of the female genital tract responsible for this greater susceptibility. Immune activation is another key factor in HIV-1 acquisition and pathogenesis that distinguishes men and women, with women exhibiting higher levels of immune activation. Data to date have pointed toward an important role of sex hormones in mediating these sex differences observed in the manifestation of HIV-1 disease. This chapter will focus on the discussion of (1) sex differences in HIV-1 acquisition and transmission, (2) sex differences in HIV-1 pathology, and (3) the impact of sex hormones including those exogenously delivered during contraceptive use. Sex differences related to responses to antiretroviral treatment go beyond the scope of this chapter and are reviewed in Chap. 4.

Abbreviation

ADI	AIDS-defining illness
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
cART	Combination antiretroviral therapy

M. Griesbeck
Centre d'Immunologie et des Maladies Infectieuses (CIMI), Paris, France

M. Altfeld (✉)
Heinrich-Pette-Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany
e-mail: marcus.altfeld@hpi.uni-hamburg.de

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_5

CDC	Centers for Disease Controls and Prevention
CMV	Cytomegalovirus
CTL	Cytotoxic CD8+ T lymphocytes
DMPA	Depot medroxyprogesterone acetate
ERα	Estrogen receptor alpha
FDC	Follicular dendritic cells
FSW	Female sex workers
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HESN	HIV-1-exposed seronegative
HHV-8	Human herpesvirus 8
HIV	Human immunodeficiency virus
HPV	Human papillomavirus (HPV)
HSV-2	Herpes simplex virus 2
IDO	Indoleamine (2,3)-dioxygenase
IDU	Intravenous drug users
IFI16	Gamma-interferon-inducible protein 16
IFNα	Interferon-α
IHC	Injectable hormonal contraception
IL-1β	Interleukin-1β
IL-2	Interleukin 2
IL-6	Interleukin 6
IL-12	Interleukin 12
ISGs	Interferon-stimulated genes
IUDs	Intrauterine devices
KS	Kaposi's sarcoma
LCs	Langerhans cells
LPS	Lipopolysaccharide
MDR	Multidrug resistant
MIP-3α	Macrophage inflammatory protein 3α
MIP-1α	Macrophage inflammatory protein 1α
MIP-1β	Macrophage inflammatory protein 1β
MPA	Medroxyprogesterone acetate
MSM	Men who have sex with men
MTCT	Mother-to-child transmission
MX-1	Myxovirus resistance 1
OCs	Oral contraceptives
PrEP	Pre-exposure prophylaxis
PRR	Pathogen recognition receptors
SIV	Simian immunodeficiency virus
STDs	Sexually transmitted diseases
STIs	Sexually transmitted infections
TB	Tuberculosis
TNFα	Tumor necrosis factor alpha
TLR	Toll-like receptors
USA	United States
WHO	World Health Organization

5.1 Introduction

5.1.1 Impact on Women and Men Throughout the History of the HIV-1 Pandemic

The first cases of acquired immunodeficiency syndrome (AIDS) were reported by the Centers for Disease Controls and Prevention (CDC) in the United States (USA) in 1981 among men who have sex with men (MSM) (1981), followed soon after by cases among intravenous drug users (IDU) and hemophiliacs. In 1983, the causative agent of AIDS was identified with the isolation of HIV-1 particles (Barre-Sinoussi et al. 1983). By 1983, AIDS cases were acknowledged among women. The proportion of women with AIDS in the USA rapidly increased, doubling over less than two decades (2001). In 1987, AIDS was the leading cause of death in women of reproductive age (15–44) in the USA (Chu et al. 1990). However, research during the earliest phases of the HIV-1 pandemic in the 1980s mainly focused on the most affected populations at the time in industrialized countries, MSM, and later IDU. It was only in the 1990s that it was slowly recognized that women were acquiring HIV-1 heterosexually (Harris et al. 1983; Redfield et al. 1985; PA 1988). Despite this, the specific needs of HIV-1-infected women were not addressed. Instead, research rather focused on women as potential transmitter of the diseases by emphasizing mother-to-child transmission or on commercial sex workers who could pass on HIV-1 to their clients and, through these clients, to the general population (Amaro 1995; Exner et al. 2003). In parallel, the HIV-1 pandemic started to affect Africa with dramatic proportions. Interestingly, the characteristics of the pandemic in Africa were contrasting with those of the pandemic in industrialized world: cases of AIDS were equally distributed between men and women (Piot et al. 1984; Quinn et al. 1986). In addition, heterosexual transmission was the main route of transmission in both men and women (Piot et al. 1984; Quinn et al. 1986). From that point forward, the most vulnerable victims of the HIV-1 epidemic were women from the southern hemisphere.

A second virus, HIV-2, which was first reported in West Africa in 1986 (Clavel et al. 1986) can cause AIDS. It remains largely confined to West Africa and to cohorts in European countries with links to West Africa, such as Portugal (Quinn 1994; Chang et al. 2002; Diop et al. 2000; Semaille et al. 2007). Although HIV-1 and HIV-2 share the same modes of transmission through sexual contact and blood (Adjorlolo-Johnson et al. 1994) and both result in AIDS, it is generally acknowledge that HIV-2 is characterized by slower rate of disease progression than HIV-1 (Jaffar et al. 2004; Marlink et al. 1994; Rowland-Jones and Whittle 2007) and is overall less transmissible (De Cock et al. 1993; Marlink et al. 1994; Adjorlolo-Johnson et al. 1994; Nyamweya et al. 2013). Dual infection by HIV-1 and HIV-2 has been described (Alabi et al. 2003). HIV-2 prevalence in West Africa has been declining in the past two decades (da Silva et al. 2008; Hamel et al. 2007; Larsen et al. 1998; Tienen et al. 2010). Because of its sporadic distribution in the world, excluding West Africa, and its less pathogenic nature, HIV-2 has been less studied,

and particularly in regards of sex differences. A few studies have reported no difference in mortality between HIV-2-infected men and HIV-2-infected women (Norrgren et al. 1998; Poulsen et al. 1997; Ricard et al. 1994; Holmgren et al. 2007). However, a higher incidence of HIV-2 infection and HIV-1/HIV-2 dual infection has been reported in women, particularly older women, than in men (da Silva et al. 2008; Holmgren et al. 2003). A higher incidence of HIV-1 in women than in men has been reported and will be addressed later in this chapter. The mechanisms responsible for this higher incidence, which may be common between HIV-1 and HIV-2, will be described later in this chapter. We will focus here on sex differences specifically related to HIV-1 infection. Given that HIV-2 represents a model of a naturally less pathogenic infection, further research describing sex differences in HIV-2 infection may provide critical insights in our understanding of the pathogenic consequences of sex differences in HIV-1 infection.

According to the UNAIDS REPORT 2013, there were worldwide about 35.3 million people living with HIV-1 in 2012. Women represent 52 % of all people living with HIV-1 in low- and middle-income countries while they represent a much lower proportion in high-income countries (2013). HIV-1 continues to profoundly affect women and girls across all regions, but the main burden is found in sub-Saharan Africa where 80 % of HIV-1-infected women live (2013). The majority of new HIV-1 infections in women in 2010 were diagnosed in women between 25 and 44 years of age. Furthermore, it is estimated that 25–30 % of all deaths in women between the ages of 25 and 40 years old worldwide were due to AIDS and tuberculosis (TB) in 2010 (Lozano et al. 2012). Heterosexual contact is the major route of HIV-1 transmission in women in developing and industrialized countries. Given the dramatic impact of the HIV-1/AIDS epidemic on women's health worldwide, it is critical to better understand the sex-specific differences in the pathogenesis and manifestations of this infection (Clayton and Collins 2014).

5.1.2 HIV-1 and Progression to AIDS

5.1.2.1 HIV-1 Life Cycle

CD4+ T cells or "T-helper cells" represent the main target cells for HIV-1 replication. These cells orchestrate the immune responses, signaling other cells of the immune system to perform their special functions. HIV-1 binds to its target cells through interactions with CD4 and co-receptors such as CCR5 or CXCR4, depending on the viral strains. HIV-1 can then fuse with the cell and release its genetic material. HIV-1 is a single-stranded RNA virus. As such, it needs to convert its RNA into DNA, which is then integrated into the host cell's genome. HIV-1 reverse transcriptase is the enzyme allowing this conversion. Upon host cell activation, the integrated DNA is transcribed into messenger RNA by the host

cell's transcription machinery in cooperation with viral genes such as *tat*, the gene product of which accelerates transcription. Genomic RNA is also transcribed for later incorporation into the budding virion. HIV-1 mRNA is then translated into a long protein precursor. HIV-1 protease is required to cut HIV-1 protein precursors that will subsequently assemble with HIV-1 RNA to form new viruses. The newly formed viruses exit the cells by budding, using part of the membrane of the cells. During different stages of the viral life cycle, host restriction factors, including APOBEC3G/3 F, TRIM5α (Huthoff and Towers 2008; Chakrabarti and Simon 2010), and tetherin (BST-2/CD317/HM1.24), can interfere with the production of new viruses (Douglas et al. 2010; Yan and Chen 2012).

5.1.2.2 Stages of HIV-1 Infection

The first phase of HIV-1 infection called "*primary HIV-1 infection*" or "*acute phase*" is characterized by a drop in CD4+ T cell numbers in the blood, termed "CD4+ T cell count" and massive production of virus that spreads throughout the body, seeding a persisting viral reservoir in various organs, particularly the lymphoid organs such as the thymus, spleen, and lymph nodes. During this acute phase of infection, up to 70 % of HIV-1-infected people suffer flu-like symptoms. Transmission risk is very high during this early phase of infection, as viral load is extremely high (Wawer et al. 2005). Within 2–4 weeks after initial HIV-1 infection, adaptive immune responses consisting of cytotoxic CD8+ T lymphocytes (CTL) and antibodies produced by B cells help reduce the levels of viral replication to a so-called *viral set point*. The viral set point is highly predictive of the rate of HIV-1 disease progression, with individuals having high levels of viremia being more likely to develop AIDS faster than those with low levels of virus. Following the initial control of acute viremia, CD4+ T cell counts can begin to increase again, but do not return to preinfection levels in most cases. The subsequent "*asymptomatic*" or "*chronic*" phase of HIV-1 infection is characterized by low levels of HIV-1 virus replication, persistent immune activation, and the absence of HIV-1-related symptoms. This phase can last for several years and even decades. The last stage of the infection is called "acquired immunodeficiency syndrome" (AIDS) and is characterized by a CD4+ T cell count below 200 cells per cubic millimeter of blood (200 cells/mm3) or by the development of one or more opportunistic illnesses regardless of CD4+ T cell count. Antiretroviral therapy (ART) can prevent disease progression to AIDS and results in immune reconstitution and increases in the CD4+ T cell count in treated individuals. While the time between HIV-1 infection and the development of AIDS has been shown to be similar between women and men, the manifestations of the infection differ between the sexes, with women developing a lower viral set point during the initial phase of the infection. Figure 5.1 summarizes the different phases of HIV-1 infection and offers a quick snapshot of sex differences across the different stages of infection.

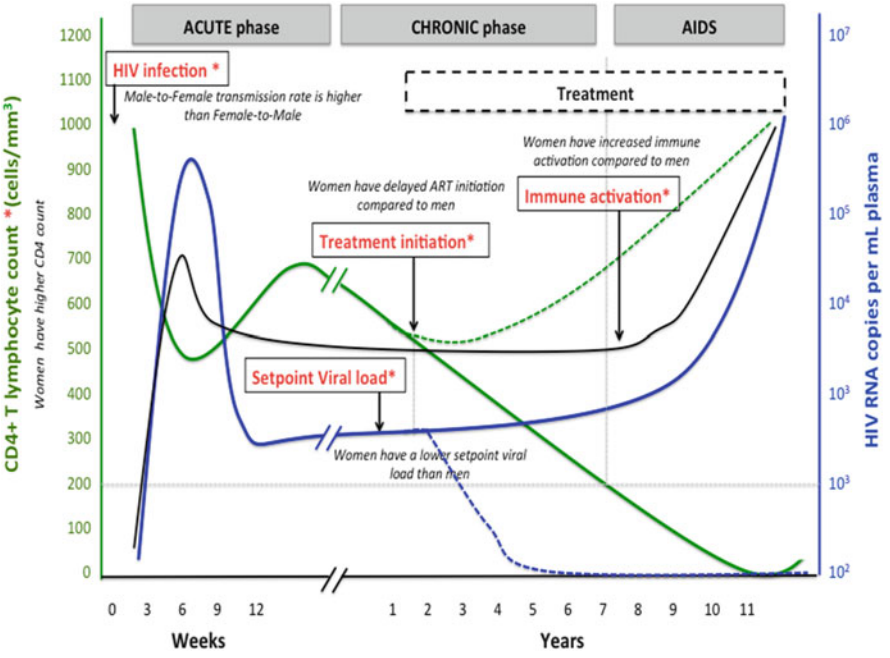

Fig. 5.1 Sex differences have been reported throughout the course of HIV-1 infection. Male-to-female transmission rate is higher than female-to-male transmission rate. Immunological and virological characteristics also appear to differ between the sexes with women generally having higher CD4+ T cell counts and lower viral set point than men. These differences may lead to delayed initiation of antiretroviral therapy (ART) in women in regard to earlier guidelines. Women also have increased immune activation compared to men, which could impact the occurrence of AIDS- and non-AIDS-related events

5.1.3 Do Gender Inequalities Drive HIV-1 Epidemic? Socioeconomic Considerations

Vulnerability to HIV-1 acquisition is defined by the negative influence of factors outside the control of the individual on their ability to protect themselves against HIV-1 infection risk. Women often represent a more vulnerable population to HIV-1 acquisition, especially in African nations and other resource-poor countries. HIV-1-positive individuals are frequently exposed to *stigmatization*, notably for being considered as socially marginalized (i.e., homosexual, hypersexual, prostitute, or drug user). Some studies have suggested that discrimination can be stronger against HIV-1-infected women as they might be seen as responsible for the spread of the virus (Gray and Berger 2007). Furthermore, women in developing countries often lack control over their sexuality and sexual relationships. *Coercive sex* including cultural/economic obligations to have sex when it is not really wanted, sexual assaults, as well as harmful cultural practices that include genital mutilation and practices such as "dry" sex increases women's risk of becoming infected with

HIV-1 (Garcia-Moreno and Watts 2000; Krug et al. 2002; Gray and Berger 2007). Two recent studies have demonstrated that women who have experienced intimate partner violence were 50 % more likely to have acquired HIV-1 than women who had not experienced violence (Jewkes et al. 2010; Kouyoumdjian et al. 2013). In a study from Kenya, a country of high rates of HIV-1 prevalence, rape accounted for 4 % of HIV-1 infection in adolescents and over their lifetime, 24 % of women (15 years and above) in Kenya reported to be raped at least once (Muntemba et al. 2003). From a physiological perspective, sexual violence causes genital injury and extragenital trauma in 87–92 % of victims (Slaughter et al. 1997), causing systemic and local inflammation and increased presence of target cells for HIV-1 at the site of exposure, which in turn can increase susceptibility to HIV-1 acquisition. Sexual assault might furthermore increase the risk of HIV-1 due to forced anal intercourse, concurrent transmission of other STIs, as well as inability to negotiate condom use. Reducing violence against women therefore remains a major challenge identified by UNAIDS for HIV-1 prevention (UNAIDS 2013). In addition, women are at higher risk for insufficient *health management*, as women often serve as the main caregivers among their family. Therefore, they might neglect their own health needs, having, for instance, less time to keep outpatient appointments, in favor of those for their husband or children. Clinical management is also often based on research performed on men and might therefore not meet women's specific needs (Sandelowski et al. 2009). Differences in education might further increase women's, and especially young girls', risk of HIV-1 infection, due to their lack of knowledge about prevention methods. In the early phases of the HIV-1 pandemic in Africa, higher educational degree was associated with a greater risk of infection (Fylkesnes et al. 1997; Hargreaves and Glynn 2002; Smith et al. 1999). The interplay of several factors including the higher wealth and increased mobility associated with higher educational degrees could explain the aforementioned association (Kilian et al. 1999). It has now been shown in African countries that people with a secondary education, especially young women, have a reduced risk of HIV-1 infection (Michelo et al. 2006; de Walque et al. 2005). However, *societal factors* are not only relevant to HIV-1 infection for women but also for men. Men are subject to social pressures that can negatively affect their response to treatment, including the prevailing concepts of masculinity associated with a sexual risk-taking behavior. Furthermore, men and boys are more rarely engaged in major international and governmental efforts to reduce HIV-1 incidences in Africa (2012a).

Poverty has been considered an important driver of the HIV-1 pandemic and the subsequent gender inequalities associated with HIV-1 infection. In the industrialized world, women among the general population (HIV-1-seronegative and HIV-1-seropositive) tend to have lower income, be underinsured for health care, and have less access to health care including antiretroviral treatment (Chaisson et al. 1995; Melnick et al. 1994; Fleishman et al. 2012; Althoff et al. 2014). It has been shown that among the newly HIV-1-infected African American women in the USA in 2010, 84 % were living below the poverty line (Ivy et al. 2013). The association between HIV-1 prevalence and economic status in Africa is controversial (Hargreaves et al. 2007; Lopman et al. 2007; Mishra et al. 2007) due to the complex interrelationships between wealth and urbanism (Hargreaves et al. 2007; Lopman

et al. 2007; Mishra et al. 2007; Hajizadeh et al. 2014). In conditions of extreme poverty, women might be more likely than men to use sex as a means to procure food for themselves and their children (Zulu et al. 2002; Weiser et al. 2007).

In this chapter, we will summarize epidemiological studies that have assessed sex differences in different aspects of HIV-1 infection, mainly HIV-1 transmission and immunopathology, and highlight the potential biological mechanisms underlying these differences. While sex differences in socioeconomic factors are not the focus of this chapter, they are very relevant to the HIV-1 epidemic and will be emphasized where required.

5.2 Sex Differences in HIV-1 Acquisition and Transmission

5.2.1 Are Women More Vulnerable to HIV-1 Heterosexual Transmission?

Heterosexual transmission was the major route of HIV-1 transmission in 2012 (2012a), with 30–40 % of annual HIV-1 infections worldwide occurring through heterosexual transmission in the female reproductive tract (2008) (Hladik and McElrath 2008). The female genital tract is composed of the vaginal, ectocervical, and endocervical mucosa (cervicovaginal mucosa). Each of those sites can be infected by HIV-1, but their relative contribution to the establishment of the initial infection remains elusive (Hladik and Hope 2009). The male genital tract is the second most frequent site of HIV-1 acquisition following the cervicovaginal mucosa. Of the nearly 15 million HIV-1-infected men, an estimated 70–75 % acquired HIV-1 through vaginal intercourse (Hladik and McElrath 2008). The risk of HIV-1 acquisition depends on the number of sexual contacts with infected persons and the probability of transmission during each sex act (Peterman and Curran 1986). Epidemiological studies have estimated that the risk of HIV-1 transmission per act is relatively low, ranging from 5 in 10,000 to 26 in 10,000 per penile/vaginal act (Chakraborty et al. 2001). There is an increasing consensus that *male-to-female transmission* is more efficient than *female-to-male transmission* (Nicolosi et al. 1994, 2013). Here, we will review the data supporting women's greater physiological vulnerability to HIV-1.

5.2.1.1 Studies on Discordant Couples: Sex Differences in Heterosexual Transmission Probabilities

The number of heterosexual transmissions in women exceeds the number of male cases in industrialized countries. This could be attributed to the simple fact that in industrialized countries, more men than women have been infected and therefore can potentially infect their female partners. Studies in HIV-1-discordant couples, defined as couples where one partner is HIV-1-infected and the other partner is

HIV-1-uninfected, therefore helped in the investigation of potential differences in transmission probabilities between men and women. Prospective studies on discordant couples are based on stable (preferably monogamous) HIV-1-discordant couples followed longitudinally after diagnosis of the index partner (Fideli et al. 2001; Gray et al. 2001; Hira et al. 1997; Wawer et al. 2005). Sexual history and seroconversion of the partner are assessed prospectively. The probability of HIV-1 transmission is associated to the infectiousness of infected person and the susceptibility of the exposed and uninfected person, which are both influenced by numerous factors including behavioral, biological, genetic, and immunological risk factors of the host and the virus. Some of those potential HIV-1 cofactors might vary over time. The duration of exposure to HIV-1 is rarely known precisely. It is therefore difficult to accurately measure per-act transmission probabilities (Shiboski and Padian 1996).

In 1992, the European Study Group on Heterosexual Transmission of HIV compared female-to-male and male-to-female transmission rates of HIV-1 in 563 stable couples and identified a 1.9 times more effective male-to-female transmission than female-to-male transmission rates (1992). Male-to-female heterosexual transmission was influenced by the *type of sexual act* with HIV-1 transmission being more efficient through penile-anal contact than through penile-vaginal contact (1992, Lazzarin et al. 1991; Seidlin et al. 1993). This increased risk is believed to be linked to physical and immunological properties of the rectal mucosa, which, for instance, lacks protective humoral immune barrier present in cervicovaginal secretions (Belec et al. 1995). Female-to-male heterosexual transmission was influenced by *male circumcision*, which is associated with a reduced risk of HIV-1 acquisition (Auvert et al. 2005; Cameron et al. 1989; Lavreys et al. 1999; Weiss et al. 2000; Baeten et al. 2005; Boily et al. 2009; Powers et al. 2008). The impact of circumcision on male-to-female transmission, however, remains controversial (Gray et al. 2000; Kapiga et al. 1998; Turner et al. 2007). *Plasma viral load* (Quinn et al. 2000) and *viral load in genital secretions* (Chakraborty et al. 2001) as well as the *stage of infection* (Pilcher et al. 2004; Powers et al. 2008) and the proportions of people with advance HIV-1 disease (de Vincenzi 1994) also influence HIV-1 transmission. Interestingly, a significant interaction was found between the clinical and immunological status of the index case and the direction of transmission. The transmission from asymptomatic men was 5 times more efficient than from asymptomatic women (1992). In contrast, the rate of transmission from index cases in advanced stages of HIV-1 infection did not differ between sexes (1992).

Early studies on HIV-1-discordant couples performed in Western countries have largely agreed that HIV-1 transmission risk was higher if the index case was male (Mastro and de Vincenzi 1996; Fischl et al. 1987; Lusher et al. 1991; O'Brien et al. 1994; Padian et al. 1991; Peterman et al. 1988; Seidlin et al. 1993, 1992; Allain 1986; Johnson et al. 1989; Nicolosi et al. 1994). Results from the US and European HIV-1-discordant studies are nevertheless counterbalanced by African studies that found more variability in their results. While some studies similarly found that male-to-female transmission rates were more efficient than female-to-

male transmission rates (Carpenter et al. 1999; Senkoro et al. 2000), others found a higher risk of HIV-1 transmission if the index case was female (Hira et al. 1990) or no difference in the risk of HIV-1 transmission between sexes (Gray et al. 2001). The recent meta-analysis of Boily and colleagues revealed contrasting differences in the direction of transmission risk by segregating countries according to their income (Boily et al. 2009). Female-to-male transmission estimates for high-income countries, adjusted for HIV-1 prevalence, were about half the male-to-female rate, although the difference failed to reach significance. By contrast, in the adjusted low-income country, female-to-male and male-to-female transmission estimates were very similar. Female-to-male estimate was larger in low-income country than in high-income country. It has been suggested that the greater variability in the results obtained from studies in African countries as compared to those obtained from studies in Western countries might be linked to differences in the characteristic between HIV-1 pandemic in Africa and in industrialized countries (O'Farrell 2001; Higgins et al. 2010). O'Farrell suggested that the higher female-to-male HIV-1 transmission rates observed in developing countries could be attributed to the higher prevalence of sexually transmitted infections (STIs), but might also be linked to the lower proportions of men circumcised (O'Farrell 2001).

As mentioned above, the probability of transmission also depends on the *infectiousness of the HIV-1 infected person*. Quinn and colleagues noticed from their studies on HIV-1-discordant couples in Uganda that the mean serum HIV-1 RNA level of the index partner in couples in which the HIV-1-negative partner seroconverted was significantly higher than that of the index partner in couples in which the HIV-1-negative partner remained seronegative (Quinn et al. 2000). Plasma viral load correlates with viral load in seminal plasma (Gupta et al. 1997) and cervical secretions (Hart et al. 1999; Iversen et al. 1998). HIV-1-infected women have been shown to have lower viral loads than male subjects matched for age and CD4+ T cell count (Farzadegan et al. 1998; Sterling et al. 2001). Quinn and colleagues observed that these sex differences in plasma viral load were the greatest among the subjects who transmitted the virus to their partners (Quinn et al. 2000). It can therefore be hypothesized that the lower efficiency of female-to-male transmission compared to male-to-female transmission observed in early studies on HIV-1-discordant couples (pre-ART era) might be due to the lower infectiousness of HIV-1-infected women who have lower viral loads. However, male-to-female or female-to-male transmission rates within strata of viral load appeared to be similar (Quinn et al. 2000). A large longitudinal study of 3,400 African HIV-1-discordant heterosexual couples by Hughes and colleagues was the first to estimate HIV-1 infectivity after adjusting for time-varying plasma HIV-1 RNA (Hughes et al. 2012). This cofactor appears to be of great importance as each $\log_{10}$ increase in plasma HIV-1 RNA increased the per-act risk of transmission by 2.9-fold. In a model that included only condom use and sex, the estimated risk of unprotected male-to-female transmission was significantly higher than the risk of female-to-male transmission. However, after adjustment for plasma HIV-1 RNA, herpes simplex virus (HSV) 2 status, and age of the uninfected partner, similar risks for transmission from male-to-female and female-to-male were observed.

The authors concluded that the higher risk of male-to-female transmission was largely due to higher viral loads in men. Therefore, sex differences in heterosexual transmission probabilities might be linked to higher infectiousness of men. However, in the post-ART era, it is important to consider the minimal risk of horizontal HIV-1 transmission in the context of full viral suppression (Porco et al. 2004). While viral particles can be detected in the genital secretions of 5–48 % of patients with undetectable plasma viremia (Marcelin et al. 2008; Sheth et al. 2009, 2012), the risk of HIV-1 heterosexual transmission from an HIV-1-positive individual under ART with full viral suppression is extremely low (Cohen et al. 2012; Dieffenbach 2012; El-Sadr et al. 2011; Loutfy et al. 2013). The landmark HIV Prevention Trials Network 052 Study, a multicenter, randomized controlled trial monitoring 1,763 HIV-1-discordant couples showed that early initiation of combination ART (cART) was associated with a 96 % reduction in the number of linked HIV-1 transmissions relative to delayed cART (i.e., waiting to initiate cART when a clinical event occurred or CD4+ T cell count reduced 250 cells/mL) (Cohen et al. 2011). But while HIV-1 transmission from fully suppressed index cases is very low, it is important to highlight that HIV-1-infected individuals are particularly at risk of transmitting the virus during primary HIV-1 infection when viremia is high and HIV-1 status is frequently unknown.

In brief, epidemiological studies have highlighted that sex differences in viral load represent an important biological factor and might contribute significantly to sex differences in HIV-1 transmission. We will discuss below additional biological mechanisms that could render women more susceptible to HIV-1 acquisition (Fig. 5.2).

5.2.1.2 Biological Perspective of Sex Differences in HIV-1 Susceptibility

Mechanisms of HIV-1 transmission through women's genital mucosa. HIV-1 virions must traverse the cervicovaginal epithelium to reach their target cells. Several pathways, including transcytosis, endocytosis or productive infection, and transmigration via infected donor cells might be involved [reviewed in (Hladik and McElrath 2008; Tebit et al. 2012)]. Regardless of the mode, the penetration of virus through the cervicovaginal epithelium in vivo occurs rapidly within 30–60 min of exposure, as shown in SIV-infected macaques (Hu et al. 2000). Once within the epithelium, HIV-1 encounters CD4+ T cells as well as Langerhans cells (LCs). Epidermal LCs can capture HIV-1 virions and efficiently internalize them into their cytoplasmic compartments (Hladik et al. 2007). The tropism of LCs has been shown to influence the efficiency of heterosexual transmission of HIV-1 (Soto-Ramirez et al. 1996). SIV virions can be detected in genital mucosal LCs within 24 h of intravaginal inoculation of macaques (Hu et al. 2000). As LCs exit the epithelium and migrate to secondary lymphoid organs, they transport intact virions, thereby enabling infection to spread beyond the site of viral entry (Hladik and McElrath 2008; Hladik et al. 2007; Merad et al. 2008). Genital CD4+ T cells have a central role in early infection and propagation of the virus as demonstrated by SIV

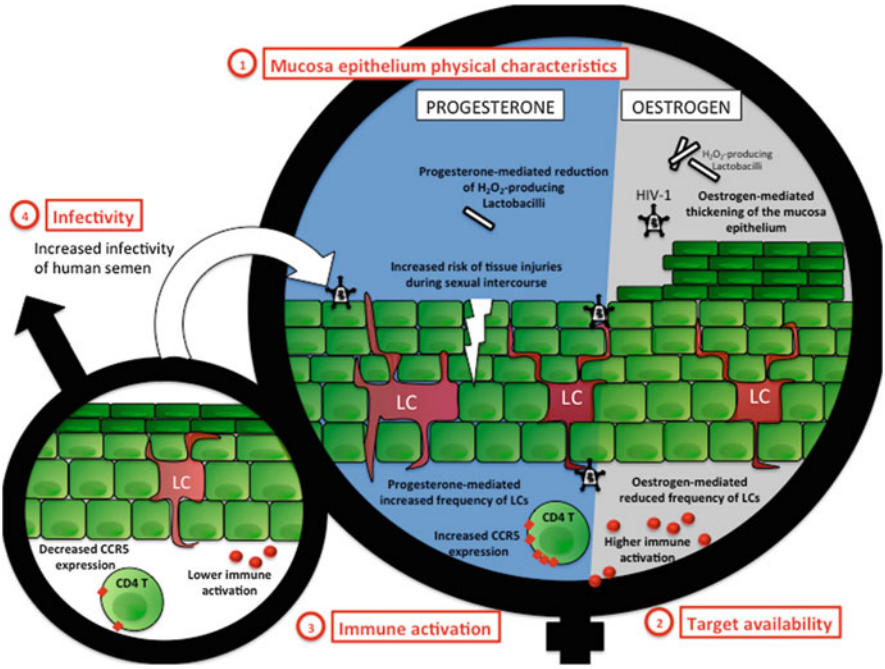

Fig. 5.2 Several mechanisms may participate in women's biological greater susceptibility to HIV-1 heterosexual transmission. The physical characteristics of the mucosa epithelium (1) including the frequency of LCs, the epithelium thickness, and the presence of H_2O_2-producing lactobacilli not only differ between men and women but are also influenced by sex hormones. Men and women may also differ in the target cell availability (2) and immune activation (3). Finally, men may be more infectious than women (4)

challenge experiments in macaques (Hu et al. 1998; Veazey et al. 2003a; Zhang et al. 1999). Within hours of infection, the founder population of infected cells is established (Miller et al. 2005a). Li and colleagues' work on SIV-infected macaque model has highlighted the exploitation of innate immune responses by SIV to overcome the limited availability of susceptible target cells to sustain and sufficiently expand the initially infected founder cell populations to disseminate and establish a self-propagating infection in secondary lymphoid organs. They demonstrate that exposure of the endocervical epithelium to the viral inoculum increases expression of macrophage inflammatory protein (MIP)-3α to recruit pDCs, which, in turn, produce MIP-1α and MIP-1β and interferon (IFN)-α to recruit CCR5 + target cells (Li et al. 2009). The founder cell populations, infected at the portal of entry, then locally expand during the first week of infection before reaching the lymphatic tissues where the spatial availability of target cells is much greater, resulting in increased viremia. The peak of virus replication in blood and tissue is thus observed during the second week after infection before declining to stable levels around four weeks post-exposure (Haase 2010).

The *mucosa of the female and male genital tracts* represents the site of initial HIV-1 exposure during heterosexual vaginal intercourse. However, their anatomic characteristics differ greatly. First, the overall surface area of mucosal HIV-1 exposure is an important *physical characteristic* to consider. Indeed, a direct association between the foreskin surface area, site of most HIV-1 acquisition in uncircumcised men, and the risk of HIV-1 acquisition has been demonstrated (Kigozi et al. 2009), suggesting that mucosa surface area and risk of HIV-1 acquisition are linked. The surface area of the cervicovaginal mucosa is larger than that of the penis and foreskin. HIV-1 virions might also have a greater access to subepithelial target cells in women than in men. The keratinized skin epithelium on the foreskin and penis is relatively impermeable (Iwasaki 2010) compared to the simple columnar epithelium (in the endocervix) and the stratified squamous epithelium (in the vagina and ectocervix), which lacks tight junctions (Blaskewicz et al. 2011). In addition, the HIV-1 mucosal exposure time within the female genital tract is longer than within the male genital tract during sexual intercourse (Bolan et al. 1999). Semen can remain within the female genital tract for up to 3 days *post-coitus* (Jain and Muralidhar 2011). Women also have an increased risk of tissue injuries during intercourse (Bolan et al. 1999). Furthermore, higher expression of the HIV-1 co-receptor CCR5 was observed on cervical CD4+ T cells from endocervical cytobrushes derived from 27 HIV-1-uninfected Kenyan women (McKinnon et al. 2011) compared to CD4+ T cells isolated from the foreskin of 46 HIV-1-uninfected Ugandan men (Prodger et al. 2012). Compared to heterosexual men, women also have greater probability of virus exposure on the rectal mucosa. In addition, factors in human semen, most notably amyloid fibrils forming from naturally occurring fragments of seminal prostatic acidic phosphatase, can capture virions and promote attachment to epithelial cells and leukocytes, thus increasing infectivity (Munch et al. 2007).

Immune activation is another feature distinguishing men and women that might account for sex differences in HIV-1 acquisition. While some studies have concluded that immune activation is protective against HIV-1 acquisition (Biasin et al. 2000; Jennes et al. 2003; Suy et al. 2007; Tomescu et al. 2010), more recent studies have shown that lower levels of immune activation in the blood correlated with reduced HIV-1 incidence and concluded that a "quiescent immune" phenotype is protective (Begaud et al. 2006; Jennes et al. 2006; McLaren et al. 2010; Pancino et al. 2010). Our analysis of the results from the CAPRISA 004 phase IIb, randomized, placebo-controlled clinical trial assessing the safety and effectiveness of 1 % tenofovir gel in preventing HIV-1 infection in women showed that women who acquired HIV-1 had significantly higher systemic innate immune activation prior to infection than women who remained uninfected, irrespective of microbicide use (Naranbhai et al. 2012). Higher cervicovaginal cytokine concentrations were also observed in women who acquired HIV-1 in the CAPRISA 004 trial (Roberts et al. 2012). In addition, studies on HIV-1-exposed seronegative (HESN) cohorts have also implicated elevated immune activation as a risk factor for acquiring HIV-1 (Card et al. 2009; McLaren et al. 2010). The female genital tract in HESN female sex workers (FSW) was characterized by immune quiescence at the mucosal

level compared to non-FSW controls. Indeed, cervical mononuclear cells from HESN had reduced expression of genes encoding proinflammatory cytokines compared to low-risk HIV-1-negative non-FSW (Chege et al. 2012). Reduced T cell activation is believed to limit the pool of activated CD4+ T target cells permissive to HIV-1 infection. In addition, low levels of proinflammatory cytokines at the infection site also prevent the recruitment of potential target cells to the site of infection (Jaspan et al. 2011). Since HIV-1 preferentially replicates in activated CD4+ T cells (Shapira-Nahor et al. 1998; Zhang et al. 1999, 2004), reduction in activation can dampen the likelihood of establishing productive infection (Haase 2010). This is supported by data from monkey models of HIV-1 acquisition demonstrating that inflammatory immune responses are exploited by the virus to attract target cells at the mucosal sites, favoring HIV-1 transmission (Li et al. 2009; Wang et al. 2005). Antiviral IFNα responses induced by intravaginal application of Toll-like receptor (TLR) 7 or 9 agonists are overcome by the virus, and inflammatory responses are used to fuel infiltration of activated CD4+ T cells and LCs at the vaginal mucosa (Wang et al. 2005). Therefore, blockade of inflammatory responses protected from infection by preventing attraction of target cells to the genital tract mucosa (Li et al. 2009). It is well established that systemic innate immune activation is in general higher in women than men (Fish 2008). Although a sex difference in immune activation at the genital mucosa has not been reported, it has been shown that women have higher gut mucosa immune activation than men (Sankaran-Walters et al. 2013). Women might therefore be more susceptible to HIV-1 acquisition due to higher numbers of activated target cells at the site of viral transmission.

5.2.1.3 Sexually Transmitted Infections Facilitate HIV-1 Transmission and Acquisition

The higher susceptibility to HIV-1 acquisition in people with pre-existing sexually transmitted diseases (STDs) has long been noted (Fleming and Wasserheit 1999; Gray et al. 1999; Grosskurth et al. 1995; Laga et al. 1993; Piot and Laga 1989; Reynolds and Quinn 2005; Weber et al. 1986); it was not clear whether the susceptibility was due to behavioral factors or to specific biological factors that enhance susceptibility to HIV-1 (Fleming and Wasserheit 1999; Rottingen et al. 2001; Sexton et al. 2005). Numerous STIs have been associated to increased risk of HIV-1 acquisition and transmission [reviewed in Mayer and Venkatesh (2011)], such as *Trichomoniasis vaginalis* (Cu-Uvin et al. 2002; Kissinger et al. 2008; Magnus et al. 2003; Niccolai et al. 2000; Laga et al. 1994; Hughes et al. 2012; Mavedzenge et al. 2010; McClelland et al. 2007; Quinn et al. 2000) or HSV-2 which of all genital ulcer diseases shows the strongest association with HIV-1 acquisition (Brown et al. 2007). As much as 50 % of new HIV-1 infections in populations with a high prevalence of HSV-2 have been attributed to HSV-2-mediated enhancement of susceptibility (Reynolds et al. 2003; Freeman et al. 2006; Shepherd et al. 2003; Wald and Link 2002). Furthermore, HIV-1-

infected individuals who are coinfected with STDs are more likely to transmit HIV-1 to their sexual partners than mono-infected individuals (Cameron et al. 1989). Indeed, perturbations of the vaginal microflora caused by STDs are associated with increased HIV-1 expression in the genital tract (Cu-Uvin et al. 2001; Rotchford et al. 2000), and HIV-1 RNA in semen of HIV-1-infected men is increased in the context of urethritis, defined as the inflammation of the tube connecting the urinary bladder to the genitals (Cohen et al. 1997). STDs appear to increase HIV-1 transmission and acquisition by several different mechanisms. By causing lesions in the mucosa, STDs might compromise mucosal integrity (Figueroa et al. 1994; Guimaraes et al. 1997; Padian et al. 1990). In addition, STDs increase inflammation resulting in higher levels of target cells for HIV-1 at the mucosal site (Kaul et al. 2008; Rebbapragada et al. 2007; Zhu et al. 2009; Pudney et al. 2005). For instance, *Neisseria gonorrhoeae* activates TLR2 leading to the activation of resting CD4+ T cells (Ding et al. 2010) but also enhances the ability of activated dendritic cells to present HIV-1 to other susceptible cells (Zhang et al. 2005). Ulcerative STDs such as syphilis, HSV-2, and chancroid might also increase HIV-1 concentrations in genital lesions, semen, or both by favoring its replication in vivo through the induction of proinflammatory cytokines (Cohen et al. 1997; Ghys et al. 2007; Johnson and Lewis 2008; Kissinger et al. 2009; Wang et al. 2001). The effect of STDs on HIV-1 transmission and acquisition seems to be long lasting, as residual inflammation might slow the return of the normal genital tract milieu to a pre-STD state. Higher HIV-1 concentration in genital tract secretions compared to HIV-1-infected individuals without STDs can persist in HIV-1-infected individuals with *gonorrhea* even two weeks after treatment for urethritis (Cohen et al. 1997).

In addition, STI coinfections might have an important contribution to the described sex-specific differences in HIV-1 susceptibility. First, the prevalence of some STIs, such as *Chlamydia trachomatis* or HSV-2, is higher among women than men in the USA and also in Africa (Quinn and Overbaugh 2005; Weiss et al. 2001; 2010b) and is substantially greater in younger women (14–19 years) than in women aged 29 years or older (Datta et al. 2012; Weiss et al. 2001). Similar transmission directions (increased male-to-female transmission rate as compared to female-to-male transmission) have been observed for many sexually transmitted diseases such as *gonorrhea*. The presence of genital ulceration has been shown to increase the per-sex act probability of HIV-1 transmission 50–300 times for female-to-male transmission (Fleming and Wasserheit 1999; Laga et al. 1993; Rottingen et al. 2001; Hayes et al. 1995). Thus, in countries with high STI prevalence, the higher effect of STDs on female-to-male transmission could counterbalance the higher risk of transmission from men to women in the absence of STDs.

5.2.1.4 High Risk of HIV-1 Acquisition in Young Women (15–24 years)

Worldwide, 76 % of young people (aged 15–24 years) living with HIV-1 are female, and vaginal intercourse remains the most prevalent route of infection

(2008). Many young women are infected after just a few sexual experiences (Glynn et al. 2001). It has been suggested that the risk of infection might be very high during the first episode of sexual intercourse for women associated with loss of virginity (Bouvet et al. 1989; Johnson et al. 1989). Furthermore, a number of differences that might be very relevant for the risk of HIV-1 acquisition exist between the genital mucosa of adolescent and women of reproductive age postpuberty (Farage and Maibach 2006), including the nature of the cervicovaginal epithelium (Critchlow et al. 1995; Gray-Swain and Peipert 2006). Cervical ectopy, defined as the extension of the columnar epithelium beyond the endocervix to include the ectocervix, is frequently present in young women (aged 15–24 years) (Critchlow et al. 1995; Hwang et al. 2011) and has been associated with a greater risk of HIV-1 acquisition (Mati et al. 1995; Moscicki et al. 2001; Moss et al. 1991; Plourde et al. 1994). Adolescents (aged 15–19 years) have also been reported to be more prone to genital microabrasions during sex, providing a portal for HIV-1 entry (Stanley 2009). As described above, an inflamed genital milieu has been associated with an increased risk of HIV-1 acquisition in several studies (Levinson et al. 2009; Roberts et al. 2012). Genital secretions of young women (15–24 years) have increased levels of proinflammatory cytokines, and young women also have more inflammatory immune cells present in cervicovaginal lavage fluids (Ghanem et al. 2005; Hwang et al. 2011). Taken together, these data suggest that the physiological and immunological characteristics of the cervicovaginal epithelium of young women might render them particularly at risk for HIV-1 acquisition.

5.2.2 Sex Differences in the Genomic Diversity of Transmitted Viruses

During HIV-1 transmission, the transmitted virus is exposed to a significant bottleneck, and only a single virus or very few viruses of the highly diverse viral population in the donor establishes an infection in the new recipient. Early studies have reported that the virus population isolated from the blood of heterosexually infected men during acute infection was very homogeneous, whereas the virus population found early in infection in women was more heterogeneous (McNearney et al. 1992; Wolfs et al. 1992; Zhang et al. 1993; Zhu et al. 1993; Kampinga et al. 1997; Poss et al. 1995). Given that the studies on infected men were conducted in North America and Europe and the ones on women in Africa, it has been suggested that geographical differences in the nature of the circulating viruses might have biased this observation. Long and colleagues compared HIV-1 envelope gene sequences in recently infected men from Kenya to sequence data from an earlier study in FSW from the same African cohort (Poss et al. 1995; Long et al. 2000). Women, at least in that study, were found to be infected with more genetically diverse viruses than men (Long et al. 2000). Follow-up reports have shown that viral diversity was not restricted to male-to-female transmission (Sagar

et al. 2004a; Ritola et al. 2004) and the percentages of men and women infected by a heterogeneous virus population in North America appeared to be similar (Sagar et al. 2004a). Chomont and colleagues noticed that viral sequences were similar in the female genital compartment and in the plasma during primary infection, but became distinct in chronic infection (Chomont et al. 2007), suggesting that reduction in genetic diversity must occur at the portal of entry before dissemination. The group lead by Eric Hunter has highlighted the major restrictive role of the integrity of the mucosal barrier on sexual transmission (Derdeyn and Hunter 2008). They showed that in the presence of inflammatory STDs, more than one genetic variant can establish infection. Therefore, in situations in which the mucosal barrier is compromised, multiple variants can be transmitted and are capable of replicating in the new host (Haaland et al. 2009; Salazar-Gonzalez et al. 2009).

Potential differences in the biology of sexual transmission between viral subtypes (Derdeyn and Hunter 2008; Chohan et al. 2005; Derdeyn et al. 2004; Frost et al. 2005) might also affect the sex differences in the diversity of the transmitted viruses. A study from Njai and colleagues in Tanzania showed that compared with men, women were less likely to have subtype D versus A (Njai et al. 2013). Women with subtype C had higher genital tract viral load compared to women with subtype B and men with subtype C or B. (Fiscus et al. 2013). A very recent study determined equally low intraindividual genetic diversity in both sexes in clade B-infected individuals in Brazil (Gouveia et al. 2014). Given that sex steroid hormones have variable effect on replication or transmission of different subtypes (Ragupathy et al. 2013), they might contribute to the sex differences observed in the diversity of transmitted virus. Altogether, these data suggest that potential differences in the diversity of transmitted virus exist between men and women and might be related to factors linked to women's overall increased susceptibility to HIV-1 infection including physical characteristics of the mucosal barrier and susceptibility to STDs.

5.2.3 Sex Differences in Vertical Transmission

The risk of mother-to-child transmission (MTCT) of HIV-1 was about 10 % in low- and middle-income countries in 2012, a percentage that has decreased significantly in the past 5 years (2013). MTCT of HIV-1 can occur in utero (20–25 %), *intrapartum* (65–70 %), or postnatally (10–15 %) through breastfeeding (De Cock et al. 2000). Maternal viral load is a risk factor strongly associated with perinatal infection (Jackson et al. 2003; Mock et al. 1999; Taha et al. 2003). Therefore, antiretroviral therapy resulting in suppression of maternal viral loads greatly improved prevention of MTCT. A number of studies have shown that significantly more girls than boys are infected with HIV-1 at birth in Africa and Europe (Taha et al. 2005; Thorne and Newell 2004; Temmerman et al. 1995), even after adjustment for maternal levels of viremia and delivery factors. Data from the European Collaborative Study suggest that there might also be a sex difference in the timing of acquisition, with girls being more susceptible to HIV-1 acquisition in utero

versus *intrapartum* than boys (Thorne and Newell 2004). Indeed, differences according to the sex of the baby were observed only when infants were delivered through elective cesarean section (i.e., before membrane rupture) and not when born vaginally or by emergency cesarean (Thorne and Newell 2004). In the latter case, infants would have on average one additional week of intrauterine exposure as well as *intrapartum* exposure to infective maternal blood and genital secretions after membrane rupture and maternal–fetal microtransfusions during uterine contractions (Thorne and Newell 2004). It has been shown that the transmission rate among women undergoing elective cesarean section is significantly lower than that among women having either nonelective cesarean section or vaginal delivery (1999). In addition, female infants continued to acquire HIV-1 infections postnatally through breast milk more frequently than boys until the age of 6 to 8 weeks, but this difference did not reach statistical significance (Taha et al. 2005). Similarly, HIV-1 seroprevalence at 15 months of age was shown to be significantly higher among malnourished girls than boys (Beau and Imboua-Coulibaly 1999). Galli and colleagues compared the male/female ratios of HIV-1-infected children born in 1985–1995 and in 1996–2001 in Italy. Interestingly, the effect of sex was present only in children born after 1995 (Galli et al. 2005). They hypothesized that this might be related to the introduction of preventive interventions, such as elective cesarean section or maternal antiretroviral regimens in pregnancy. However, the higher risk of infection observed in female infants compared to male infants was independent of the type of delivery and administration of antiretroviral regimen to the mother (Galli et al. 2005). Biggar and colleagues studied HIV-1 transmission between sex-discordant twins and also found that female twin was more likely to be infected than the boy (Biggar et al. 2006).

The higher HIV-1 infection rates of female infants can be explained either by a higher susceptibility to HIV-1 infection in female than male infants or alternatively by higher in utero mortality rates of HIV-1-infected male infants. The hypothesis of higher in utero mortality in male infants is supported by the male-to-female sex ratio of births from HIV-1-infected mothers of 0.98:1.00 observed (Taha et al. 2005), which is lower than the typical male-to-female ratio of 1.03:1.00 expected in Africa and elsewhere (Garenne 2002). Generally in developed countries, male fetuses have a higher rate of spontaneous abortion (Byrne and Warburton 1987), but more boys than girls are conceived as well, so that about half of live births are boys (Biggar et al. 1999). In utero and delivery-related HIV-1 infections probably occur mainly because of microtransfusions of infected maternal lymphocytes across the placenta. H-Y incompatibility cellular reactions occur when maternal cells are microtransfused into boys and provide supporting arguments that H-Y incompatibility reaction initiated by maternal cells could explain the excess risk of mother-to-child HIV-1 transmission in girls. Transplantation studies have revealed that female cells react to Y chromosome-derived antigens (Uphoff 1975; Gratwohl et al. 2001; Spierings et al. 2003; Urbano-Ispizua et al. 2002), which could result into some degree of protection against HIV-1 acquisition in boys. Indeed, maternal lymphocyte reactions to Y chromosome-derived antigens could reduce infection risk for boys by the release cytokines that either block HIV-1

infection or inhibit HIV-1 replication or by shortening maternal lymphocyte survival in boys through maternal lymphocyte Y antigen-induced cell death.

However, other studies in the USA (Connor et al. 1994) and Africa (Guay et al. 1999; Coutsoudis et al. 2004) failed to observe increased risk of HIV-1 among girls. Those studies had relatively few infected infants and did not differentiate in utero versus perinatal risk. A meta-analysis based on African studies reported that boys were at significantly greater risk of infection via breastfeeding than girls at 4 weeks of age (Coutsoudis et al. 2004). Although the authors controlled for similar duration of breastfeeding between boys and girls, they could not account for potential cultural bias that would presume that boys may be more (differences in the total daily volume of milk ingested) or more frequently breast-fed. The authors were not able to control for the type of feeding either (exclusive breastfeeding vs. mixed breastfeeding), with mixed breastfeeding having been associated with a greater risk of transmission of HIV-1 (Coutsoudis et al. 2001; Smith and Kuhn 2000). Piwoz and colleague did not observed an effect of sex on postnatal transmission after adjustment for the type of breastfeeding (Piwoz et al. 2006).

In conclusion, the HIV-1 infection rate in utero appears to be greater in girls than boys and might be due to either protection in boys through immunological responses to the Y chromosomes by maternal cells mother or to increased in utero mortality rates of HIV-1-infected male infants.

5.3 Sex Differences in HIV-1 Pathology

5.3.1 Sex Differences in Viral Load and Immunopathology

5.3.1.1 Sex Differences in CD4+ T Cell Counts

HIV-1 infection is characterized by a continuous decline in CD4+ T cell counts, eventually leading to immunodeficiency and AIDS. Results from studies assessing sex differences in CD4+ T cell counts have been discrepant. While some studies have reported higher CD4+ T cell counts in HIV-1-infected women than in men (Collazos et al. 2007; Loupa et al. 2006; Moore et al. 2003; Nicastri et al. 2005; Prins et al. 1999; Mocroft et al. 2000), others have not observed any difference according to sex (Finkel et al. 2003; Saves et al. 1999; Sterling et al. 2001), and one study reported lower CD4+ T cell counts in women than in men (Moore et al. 2002). Differences in these studies are most likely due to differences in the stage of HIV-1 infection at which the CD4+ T cell counts were assessed. In the pre-ART era, women have been reported to have higher CD4+ T cell counts at seroconversion with a difference of about 100 x 10^6 cells/l between HIV-1-infected men and women shortly after HIV-1 infection (1994; Delmas et al. 1997), but also at AIDS and death (Prins et al. 1999). Of note, higher CD4+ T cell count in HIV-1-uninfected women compared to HIV-1-uninfected men also has been noted (Maini

et al. 1996; Tollerud et al. 1989, 1994, Reichert et al. 1991), suggesting that baseline sex differences have to be taken into account when the significance of a CD4+ T cell count is evaluated among infected individuals. Similarly, studies evaluating changes in CD4+ T cell counts over time in women and men have lead to conflicting results. Some studies have reported similar diminutions in CD4+ T cell counts following infection in men and women (Prins et al. 1999; 2003; Delmas et al. 1997), while others observed a faster CD4+ T cell count decline in women (Greenspan et al. 2000). Studies on sex differences in CD4+ T cell count recovery after initiation of ART have also been controversial. While some studies did not find differences according to sex (Thorsteinsson et al. 2012; Mocroft et al. 2000; Nicastri et al. 2005; (Moore et al. 2003), many others have reported better immunological outcome in women than in men (Barber et al. 2011; Collazos et al. 2007; Currier et al. 2010; Zaragoza-Macias et al. 2010; Hunt et al. 2003a; Finkel et al. 2003). Greater T cell recovery in women than men was also observed in developing countries, with sex-based differences increasing with time on ART (Blacker 2004; Nash et al. 2008). Bosch and colleagues showed a greater influence of pretreatment viral RNA level on CD4+ T cell count increases in women than men (Bosch et al. 2007). The factors responsible for these sex differences in CD4+ T cell count are insufficiently understood but might be related to differences in the level of immune activation in HIV-1-infected women and men, as discussed further below.

HIV-1-infected women have significantly higher CD4+/CD8+ T cell ratio than HIV-1-infected men due to lower CD8+ T cell count (Ballesteros-Zebadua et al. 2013). The CD4+/CD8+ T cell ratio has been shown to predict the risk of both AIDS and non-AIDS-related morbidities (Serrano-Villar et al. 2013; Buggert et al. 2014), with higher CD4+/CD8+ T cell ratio associated with better prognosis (Krantz et al. 2011; Forbi and Agwale 2009). However, basal counts of CD8+ T cells appeared to be lower in healthy women than in healthy men (Ray et al. 2006). The lower CD4+/CD8+ T cell ratio observed in HIV-1-infected men might therefore only reflect baseline differences between men and women.

5.3.1.2 Sex Differences in HIV-1 Viral Load

The majority of studies assessing sex differences in viral loads have shown lower viral loads in women compared to men despite some results arguing that there are no sex differences (Bush et al. 1996; Richardson et al. 2003; Soon et al. 2012). Several large studies have reported that women exhibit between 0.13 and 0.35 $\log_{10}$ (about 50 %) lower HIV-1 RNA levels early in the infection (Evans et al. 1997; Farzadegan et al. 1998; Sterling et al. 1999; Prins et al. 1999; Bosch et al. 2007; Gandhi et al. 2006; Anastos et al. 2000; Katzenstein et al. 1996; Ballesteros-Zebadua et al. 2013). These differences in viral loads persisted for several years after seroconversion (Sterling et al. 2001) before attenuating, resulting in no detectable differences in viral loads at later stages of infection (Farzadegan et al. 1998; Sterling et al. 1999, 2001). Furthermore, it is important to note that

differences in HIV-1 RNA viral load between men and women have been shown to be larger for HIV-1-infected individuals with higher CD4+ T cell counts (Gandhi et al. 2002; Napravnik et al. 2002; Ballesteros-Zebadua et al. 2013; Donnelly et al. 2005; Gilad et al. 2003). A recent study showed that viral load differences between women and men were approximately 0.2 $\log_{10}$ among persons with CD4+ T cell counts up to 300 cells/mm^3, but less than 0.1 $\log_{10}$ among subjects with a CD4+ T cell counts below 50 cells/mm^3 (Grinsztejn et al. 2011).

Viral load suppression serves as an important clinical tool as it predicts the durability of responses to combination ART and decreases the risk for the development of drug resistance (Katzenstein et al. 1996; Rachlis and Zarowny 1998). Similarly, data relating sex differences in virological responses to ART have been conflicting. While no sex differences in virological responses between men and women were reported in some studies (Fardet et al. 2006; Mocroft et al. 2000; Nicastri et al. 2005; Thorsteinsson et al. 2012), an improved viral suppression in women than in men has been described by others (Kipp et al. 2010; Moore et al. 2001; Raboud et al. 2010; 2006), even after correction for adherence (Collazos et al. 2007; Cescon et al. 2013). Possible mechanisms for improved viral suppression in women might be increased drug levels of antiretroviral drugs following standard doses in women particularly within the protease inhibitor class (Burger et al. 2002; Ribera et al. 2004) or lower pretreatment RNA as slower viral suppression has generally been associated with higher pre-ART HIV-1 RNA (Moore et al. 2003; Phillips et al. 2001).

The mechanisms underlying these sex differences in viral loads remain unknown. It has been postulated that the effect of sex hormones on chemokine receptor expression and cytokine production (Athreya et al. 1993) might influence viral replication and disease progression (Farzadegan et al. 1998). Notably, it has also been hypothesized that tumor necrosis factor-alpha (TNFα), which is associated with immune activation and increased viral replication, might be inhibited by estrogen, resulting in a lower viral load in women (Shanker et al. 1994). However, no sex differences in TNFα production upon TLR7 stimulation, which is activated by single-stranded HIV-1 RNA, have been demonstrated (Berghofer et al. 2006; Meier et al. 2009; Seillet et al. 2012). Finally, differences in IFNα production between women and men, as discussed further below, might have a direct effect on the observed sex differences in viral loads, as higher levels of IFNα production in women might lead to enhanced expression of IFNα-stimulated antiviral host restriction factors.

Lower viral load measured in plasma does not imply higher levels of CD4+ T cell counts in infected women. This inconsistency might be linked to tissue redistribution (Ballesteros-Zebadua et al. 2013). Throughout the course of the infection, follicular dendritic cells (FDCs) trap and retain large quantities of viral particles in the germinal centers of all secondary lymphoid tissues (Keele et al. 2008; Smith-Franklin et al. 2002), which therefore represent a major viral reservoir in the body. Levels of virus in this compartment exceed by two orders of magnitude the viral load in peripheral blood (Haase 1999; Haase et al. 1996) so that the levels of virus measured in the blood only partially reflect the complex HIV-1 dynamics in the

lymphoid compartment. The lower viremia observed in females could therefore also be explained by a higher viral clearance rates, which might arise from greater immune reactivity observed in female patients (Meier et al. 2009; Shanker et al. 1994; Verthelyi 2006), or a lower transfer rate of virus released from lymphoid tissue into the blood. As virus trapping and retention on FDCs are mediated primarily by specific antibodies and/or complement proteins coupled with immune complex receptors on FDCs (Stoiber et al. 1997; Smith-Franklin et al. 2002; Kacani et al. 2000; Heath et al. 1995), the more elevated titers of neutralizing antibodies against HIV-1 observed in women compared to men (Gilbert et al. 2010; (Gilbert et al. 2005) might at least partially be responsible for the lower viremia in women. Besides, the destruction of lymphoid tissue architecture with release of large quantities of virus at the latest stage of disease (Haase et al. 1996) might account for the observation of high (and similar) viral loads in men and women at later stages of HIV-1 disease when the CD4+ T cell count is below 50 cells/mm^3. The mechanisms responsible for the described sex differences in viral loads require further investigation. A role of sex hormones in this process is supported by the observation that the CD4+ T cell count does not differ between men and women over the age of 50 (Maini et al. 1996; Tollerud et al. 1989) and that viral load can vary during the menstrual cycle (Greenblatt et al. 2000). However, given that lower viral loads are also seen in girls compared to boys (2002), it appears that sex hormones alone cannot account for all the sex differences in viremia and that more complex mechanisms, including X-chromosomally encoded genes, are most likely involved.

5.3.2 Sex Differences in Immune Activation

HIV-1 infection is characterized by a persistent activation of the immune system, with detrimental consequences. Immune activation contributes to the continuous maintenance of a pool of activated target CD4+ T cells for HIV-1 replication and spread. Immune activation might also contribute to the bystander apoptosis of uninfected CD4+ T cells and an increased production of cytokines that not only might increase HIV-1 replication but also have other deleterious effects, including accelerated immune aging or increased cardiovascular morbidity in HIV-1 infected individuals. Immune activation is strongly associated with the rate of disease progression in HIV-1-infected individuals (Lawn et al. 2001). In some studies, immune activation has been found to be a better correlate of clinical disease progression than CD4+ T cell count or HIV-1 RNA levels (Hazenberg et al. 2003; Giorgi et al. 1999). Furthermore, when compared with uninfected controls, elevated immune activation persists in HIV-1-infected individuals on ART even when HIV-1 viremia is fully suppressed (Hunt et al. 2003b; El-Sadr et al. 2006). Elevated immune activation in HIV-1 infected individuals contributes to increased risk of serious non-AIDS-related morbidity and mortality, such as cardiovascular disease, kidney disease, liver disease, and non-AIDS-defining

malignancies (El-Sadr et al. 2006; Lekakis and Ikonomidis 2010; Lichtenstein et al. 2010; Ho et al. 2010; Baker and Duprez 2010). High immune activation levels can be enhanced directly by HIV-1 replication and indirectly through changes in the microbiome, microbial translocation, coinfection with pathogens including cytomegalovirus (CMV), immune deregulation, and lymphoid tissue fibrosis (Biancotto et al. 2007; Brenchley et al. 2006; Gonzalez et al. 2009; Jiang et al. 2009). Chronic immune activation is characterized by the upregulation of many inflammatory markers, including increased expression of HLADR, CD38, and Ki67 on CD4+ and CD8+ T cells, caused in part by antigen-specific T cell activation, but mostly by bystander activation resulting from the general activation of innate immune responses.

5.3.2.1 Innate Sensing of HIV-1

Innate immunity is the first line of defense against invading pathogens and requires recognition by pathogen recognition receptors (PRR) including the TLR family. Excessive activation of these receptors and dysregulation of their signaling pathways might alter immune responses and contribute to chronic immune activation. It has been shown that HIV-1 infection modulates the TLR responses by altering levels of TLR expression and dysregulating responses of innate immune cells to TLR stimulation (Bosinger et al. 2004; Heggelund et al. 2004; Lester et al. 2008; Martinson et al. 2007; Mureith et al. 2010; Nordone et al. 2007). In the course of HIV-1 life cycle, several intermediates including intermediates resulting from abortive infection are generated, such as double-stranded DNA (Boasso and Shearer 2008). Those oligonucleotides can engage various sensors (Lee et al. 2013). HIV-1 also encodes for multiple TLR7/8 ligands (Beignon et al. 2005). TLR7/8 detects single-stranded RNA in the endosomes. It has recently been shown that the gamma-interferon-inducible protein 16 (IFI16) recognizes HIV-1 intermediaries resulting from abortive infective in lymphoid quiescent CD4+ T cells (Monroe et al. 2014). Levels of IFI16 in HIV-1-infected individuals were also correlated to chronic immune activation (Nissen et al. 2014). Levels of IFI202, the gene encoding for the murine homolog of IFI16, have been shown to be higher in splenic cells derived from female mice than in cells derived from male mice and to be regulated by sex hormones (Panchanathan et al. 2009). In addition, stimulation of innate immune cells is not limited to HIV-1-encoded ligands. HIV-1-mediated depletion of gut-associated CD4+ T cells in early in HIV-1 infection contributes to an increase in microbial translocation through a compromised mucosal barrier. The resulting elevated systemic levels of microbial products such as lipopolysaccharide (LPS) can stimulate a cascade of cytokine production via TLR4 (Brenchley et al. 2006; Jiang et al. 2009) and are associated with a general increase in immune activation (Brenchley et al. 2006).

5.3.2.2 Sex Differences in pDC IFNα Response

Persistent production of type I IFNs by pDCs has been shown to be a mechanism contributing to chronic immune activation in HIV-1 infection (Mir et al. 2011; Bosinger et al. 2011). Studies in SIV infection of rhesus macaques, the non-natural host of SIV, have shown an association between high levels of IFNα production, immune activation, and viral pathogenesis which is not observed in SIV infection of sooty mangabeys, the natural host of SIV (Jacquelin et al. 2009; Bosinger et al. 2009). Indeed, a key distinction between the two models is that innate immune activation is rapidly resolved in SIV-infected natural hosts, whereas upregulation of the type I IFN response and expression of ISGs persists in SIV-infected macaques, highlighting the potential pathogenic role of the magnitude and longevity of the IFNα response in SIV/HIV-1 disease (Harris et al. 2010; Campillo-Gimenez et al. 2010; (Jacquelin et al. 2009; Bosinger et al. 2009; Estes et al. 2008). Classically, pDCs are described as being refractory to IFNα production upon repeated stimulation with synthetic TLR7 or TLR9 agonists, which is thought to be a protective mechanism against excessive immune activation (Bjorck 2004; Ito et al. 2006). HIV-1 seems to uniquely allow for persistent stimulation of pDCs (O'Brien et al. 2011). By skewing pDCs toward a partially matured and persistently IFNα-secreting phenotype, HIV-1 might promote its replication by blunting adaptive immune responses and by inciting inflammatory responses to amplify activated target cells for infection (O'Brien et al. 2011). Excessive IFNα production by pDCs might promote HIV-1 pathogenesis through multiple distinct mechanisms, including the chemoattraction of CCR5+ CD4+ T cells at the mucosal site, therefore favoring systemic diffusion of the virus (Haase 2010), the upregulation of T cell activation markers (Boasso et al. 2008), and the induction of the immunosuppressive enzyme indoleamine (2,3)-dioxygenase (IDO), thus altering the Th17/regulatory T cell balance (Favre et al. 2010; Manches et al. 2008) [reviewed in O'Brien et al. (2013)].

Importantly in the context of sex differences in the manifestations of HIV-1 disease, it has been shown that pDCs derived from females produced markedly more IFNα in response to HIV-1-encoded TLR7/8 ligands than pDCs derived from males, resulting in stronger secondary of CD8+ T cells (Meier et al. 2009). Higher IFNα production by pDCs in response to TLR7 has also been observed for healthy women as compared to healthy men (Berghofer et al. 2006). *TLR7* is encoded on the X chromosome; however, sex differences in pDC TLR7 response do not seem to be linked to higher expression of TLR7 (Berghofer et al. 2006). Besides, the observation of a trend toward a lower frequency of IFNα-producing pDCs in postmenopausal women compared women of childbearing age suggested a role for sex hormones. The impact of sex hormones on the pDC IFNα response will be discussed later in this chapter.

It has been suggested that women have a higher risk of developing AIDS compared to men for the same level of viral replication (Farzadegan et al. 1998; Gandhi et al. 2002). Higher activation of T cells has been reported in women

compared to men after controlling for viral replication and was associated with increased IFNα production by pDCs (Meier et al. 2009). Furthermore, increased levels of a subset of interferon-stimulated genes (ISGs), including CCR5, the myxovirus resistance 1 (MX-1), and ISG15, in CD4+ T cells and CD8+ T cells from treatment-naïve HIV-1-infected women have been observed during chronic HIV-1 infection after controlling for HIV-1 viral load (Chang et al. 2013). The upregulation of those ISGs was associated with higher levels of immune activation in chronic HIV-1 infection (Chang et al. 2013). Altogether, these data suggest that sex differences in the activity of TLRs might account for higher immune activation in women compared to men at a given HIV-1 viral load and provide a mechanism by which the same level of viral replication might result in faster HIV-1 disease progression in women (Meier et al. 2009).

5.3.3 Sex Differences in Mortality and Disease Progression

5.3.3.1 Sex Differences in Disease Progression

Early studies observed a more rapid clinical progression to AIDS in infected women (Bozzette et al. 1998; Moore et al. 1999). However, these differences in disease progression were attributed to delays in starting ART, to the higher occurrence of gynecological disorders including invasive cervical cancer, and to other conditions preferentially affecting women, such as discrimination, violence, and stigma (Bozzette et al. 1998; Moore et al. 1999). Subsequent studies have found similar rates of disease progression between men and women (Cozzi Lepri et al. 1994; Junghans et al. 1999; Farzadegan et al. 1998; Sterling et al. 2001; Chaisson et al. 1995; Melnick et al. 1994; 2000; Patterson et al. 2007; Sabine 2005; Egger et al. 2002; Fardet et al. 2006; Hulgan et al. 2007; Moore et al. 2003; Murri et al. 2003), including studies after the introduction of ART in 1996 (Perez-Hoyos et al. 2003; Porter et al. 2003).

5.3.3.2 Sex Differences in Mortality

Between 1981 and 1990 in the USA, the median survival after diagnosis of AIDS was only about 14 months for men and 11 months or less for women, the latter being significantly shorter (Lemp et al. 1992; Rothenberg et al. 1987). Given that among treated individuals, the survival did not differ by sex (Lemp et al. 1992; Chaisson et al. 1995; Fabricatore et al. 2009; Friedland et al. 1991; Melnick et al. 1994), it was suggested that sex differences in survival might come from differential access to HIV-1 treatment (Lemp et al. 1992). Similarly, other studies reported that the reduced survival observed for women compared with men was due to different access to care (Melnick et al. 1994; Moore et al. 1991), differences in the utilization of therapy, or problems with adherence to complex drug regimens (Junghans

et al. 1999; Mocroft et al. 1999; Poundstone et al. 2001). These conclusions were supported by the fact that no sex differences in disease progression or survival were observed among HIV-1-infected individuals receiving medical care in the pre-ART era (Chaisson et al. 1995). Hulgan and colleagues found that sex was not a significant predictor of a new AIDS-defining illness (ADI) and death in a cohort of patients who had initiated their first ART treatment between 1997 and 2004 in the USA (Hulgan et al. 2007). However, their study only included 11 % of females. Hall et al. did not observed substantial survival differences between sexes using data from the CDC National HIV/AIDS Reporting System from 1996 to 2001. Women with HIV-1 infection had slightly higher 1-year survival rates and slightly lower rates at 3 years after AIDS diagnosis compared with men (Hall et al. 2006). In contrast, differences in survival, reflected by women having lower survival rates, were reported by Lemly et al. in the USA from 1998–2005, with the sexes having equal access to care. This difference persisted even after adjustment for duration of ART, suggesting that poorer treatment outcomes in women were not affected by duration of therapy alone (Lemly et al. 2009). Another study found that women survived longer and experienced a lower risk of both progression to AIDS and non-AIDS mortality following the introduction of ART in 1996 in industrialized countries (Jarrin et al. 2008). This appeared to be related to the longer survival of women in the general population (including uninfected individuals) of Western countries.

Higher mortality among men than women on ART in Africa has been observed in many studies (Cornell et al. 2009; Hawkins et al. 2011; Mills et al. 2011; Nglazi et al. 2011; Taylor-Smith et al. 2010; Wools-Kaloustian et al. 2006; Ferradini et al. 2006). Differences in mortality have been attributed to later presentation to ART programs by men (Stenehjem and Shlay 2008; Stringer et al. 2006; Hawkins et al. 2011; Taylor-Smith et al. 2010; Klausner et al. 2011). A recent study compared survival on ART between HIV-1-infected men and women in South Africa. HIV-1-infected men appeared to have higher mortality on ART than women (Cornell et al. 2012). This difference persisted after adjustment for measures of HIV-1 disease stages at the time of ART initiation, in the subset of patients who achieved virologic suppression, and among patients with good immune responses to treatment. Interestingly, the authors compared sex differences in mortality among HIV-1-infected patients on ART with mortality in an age-matched HIV-1-negative population and found the sex differences in mortality in HIV-1-infected individuals to be smaller than in the HIV-1-negative South African population (Cornell et al. 2012). This was explained by better access through ART services to other preventive and curative health services that reduce non-HIV-1 mortality. Increased burden of mortality among younger men due to traumatic causes and non-HIV-1 tuberculosis has been furthermore noted (Norman et al. 2007). In brief, sex differences in mortality to ART in Africa do not seem to be linked to HIV-1-specific sex differences.

5.3.3.3 Causes of Death and Sex Differences in HIV-1-Related Morbidity

The causes of death in HIV-1-infected individuals might differ according to sex (Weber et al. 2013; Hessamfar-Bonarek et al. 2010). AIDS-related deaths seem to be female-biased with women dying more frequently than men of AIDS-related causes in 2005 in France and this independently of their age (Hessamfar-Bonarek et al. 2010). In contrast, deaths related to respiratory malignancies, suicides, and accidents and, for people aged over 50 years, deaths related to cardiovascular diseases and cancers appear to be male-biased with more men dying from the aforementioned causes in 2005 in France (Hessamfar-Bonarek et al. 2010). Non-AIDS-related causes thus dominated in men (Hessamfar-Bonarek et al. 2010). Sex difference in access to care, notably due to the high percentages of migrants among HIV-1-infected women, and the more favorable risk factor profile for respiratory, cardiovascular, and violent deaths in women in general seem to be responsible for the higher proportion of deaths from AIDS in women. With ART, HIV-1-infected patients live longer (Palella et al. 1998) and die less frequently from AIDS-related diseases (Lewden et al. 2005, 2008; Palella et al. 2006; Sackoff et al. 2006). In 2010, more than half of the mortality in HIV-1-infected patients in industrialized countries was due to non-AIDS-related illnesses, including non-AIDS malignancies, non-AIDS infections, violence/drug-related causes, liver diseases, and cardiovascular diseases (2010a). With the ageing of the HIV-1-infected population, complications related to ageing, long-term exposure to treatment, cardiovascular risk factors, and chronic comorbidities are appearing (Lewden et al. 2005; Smit et al. 2006). In addition, the proportion of women aged over 50 years or older accounting for new HIV-1 cases rose from approximately 6–9 % between 2002 and 2006 in Western Europe (Clark 2005). The prevalence of comorbidities in those with HIV-1 infection appears to be similar to that observed among persons in the general population who are 10 years older.

Cancer is increasingly recognized as a complication of HIV-1 infection (Bonnet et al. 2004). The incidence rate of AIDS-defining cancers such as Kaposi's sarcoma (KS), non-Hodgkin's lymphoma, and cervical cancer is greater in HIV-1-infected individuals than in the general population (Biggar et al. 2007). Compared to the general population (Dauby et al. 2011; Goedert et al. 2006; Intra et al. 2005) of similar age (Shiels et al. 2010), HIV-1-infected women are more likely to have human papillomavirus (HPV), and the incidence of the cellular changes that precede cervical cancer is 4 to 5 times higher in HIV-1-infected women than HIV-1-negative women (Ahdieh-Grant et al. 2004; Palefsky 2009; Paramsothy et al. 2009). HIV-1 is not believed to have any direct carcinogenic effect but rather to affect cancer risk by lowering host immunity to carcinogenic infectious agents such as human herpesvirus 8 (HHV-8), which is necessary but not sufficient for the development of the KS (Clifford and Franceschi 2009; Sullivan et al. 2008). KS is one of most common cancers worldwide in HIV-1-infected individuals (Lucia et al. 2011). Its prevalence is elevated in MSM in the USA and Europe (La Ferla et al. 2013) but also in the entire population of several sub-Saharan African

countries where HHV-8 is highly endemic and not necessarily associated with HIV-1 (Eltom et al. 2002). The risk of AIDS-related KS (AIDS-KS) has been shown to be greater in HIV-1-infected men compared with HIV-1-infected women, in North America and Europe, which might be related to sexual behaviors associated with exposure to saliva (Martro et al. 2007; Casper et al. 2002, 2006). A similar observation has been made in sub-Saharan Africa (Chokunonga et al. 2013; Somdyala et al. 2010; Jie et al. 1997; McGarvey et al. 1998), where HHV-8 prevalence is equal between men and women (Maskew et al. 2011). Nevertheless, several studies have reported more aggressive KS and lower survival from KS in HIV-1-infected women than in HIV-1-infected men (Nasti et al. 1999; Mosam et al. 2012; Bohlius et al. 2014; Meditz et al. 2007; Benedetti et al. 1991; Cooley et al. 1996; Lassoued et al. 1991). Much less is known about the incidence of non-AIDS-defining cancers including kidney cancer and leukemia, but it has been suggested that their incidence was greater among HIV-1-infected men than HIV-1-infected women (Shiels et al. 2009). Altogether, this suggests that further analyses of the sex differences in both AIDS-related and non-AIDS-related comorbidities are needed to improve our understanding of their burden of disease and to develop targeted interventions.

5.3.4 Sex Differences in HIV-1 Coinfections

5.3.4.1 Hepatitis C Virus Coinfection

HCV coinfection is reported in about 30 % of HIV-1-infected individuals in the USA (Sherman et al. 2002; Staples et al. 1999). It is well established that female sex is a predictor of spontaneous clearance of acute HCV infection (van den Berg et al. 2011; Grebely et al. 2007; Micallef et al. 2006). The mechanisms behind the association of female sex and HCV spontaneous clearance might be linked to sex-based differences in immunity. For instance, it has recently been shown that the effect of the IL28B genotype on clearance was greater among women than among men (Grebely et al. 2014). This sex-based differences in immunity might also be partially responsible for the better control of HIV-1 replication in acute infection leading to the lower viral set points observed in HIV-1-infected women than in HIV-1-infected men. It has been postulated that HCV clearance in women might be facilitated by estrogens (Alric et al. 2000; Hayashi et al. 1998). However, HIV-1–HCV coinfection has been associated to HCV persistence (Grebely et al. 2007). It is unknown whether HCV clearance rate are higher in HIV-1–HCV-coinfected women as compared to HIV-1–HCV-coinfected men.

Liver-related mortality is the leading cause of death among HIV-1-infected persons in the USA in the ART era (Bica et al. 2001). It is generally acknowledged that chronic HIV-1–HCV coinfection is associated with increased mortality relative to mono-infection with either virus (Bonacini et al. 2004; Piroth et al. 2000; Soto et al. 1997) and accelerated hepatic fibrosis progression compared to patients with

HCV mono-infection (Macias et al. 2009; Ly et al. 2012; Graham et al. 2001; Sulkowski et al. 2007; Benhamou et al. 1999). In the HIV-1–HCV-coinfected population, it has been reported that women have higher mortality rates than men despite similar viral suppression and CD4+ T cell count, even when controlling for IDU history, race, and baseline CD4+ T cell count (Emery et al. 2010). The higher mortality rates in HIV-1–HCV-coinfected women were not due to sex differences in virological and immunological responses to ART (Emery et al. 2010). Sex differences in mortality could be due to more rapid HCV-related liver disease in women as suggested by Rodriguez-Torres and colleagues. They showed that HIV-1–HCV-coinfected women had a median survival time to cirrhosis of 16.0 years less than HCV-mono-infected women while there was no differences in the risk for cirrhosis between HCV-mono-infected and HCV-coinfected men (Rodriguez-Torres et al. 2006). However, those results might have been biased by an increased use of alcohol by HCV-mono-infected men (Rodriguez-Torres et al. 2006). In contrast, Collazos and colleagues have demonstrated that HIV-1–HCV-coinfected women have more favorable HCV virological and clinical profiles than men and, particularly, lower degrees of fibrosis (Collazos et al. 2011). In HCV mono-infection, it is well recognized that men have higher rate of disease progression compared to women (Poynard et al. 1997). Differences in treatment discontinuation between HIV-1–HCV-coinfected women and HIV-1–HCV-coinfected men might also potentially explain the observed sex differences in mortality rates. In HIV-1–HCV coinfection, adverse events during HCV therapy account for 12–39 % of treatment discontinuations and occur more frequently in HIV-1-infected women during HCV therapy than HIV-1-infected men (Bhattacharya et al. 2010). Generally, women including HCV-infected women and HIV-1-infected women experience more adverse events in response to drug therapies than men (Floridia et al. 2008). For instance, HCV-infected women are more likely to experience anemia (Sulkowski et al. 2004) and develop depression (Gohier et al. 2003; Koskinas et al. 2002) than HCV-infected men upon IFN and ribavirin treatment, which was until very recently the standard for HCV therapy. Depression is more frequent in HIV-1-infected women and women with viral hepatitis than men (Lipsitz et al. 1994; Rabkin et al. 1997; Semple et al. 1996; Zorrilla et al. 1996) and is an important reason for therapy interruption in women (Emery et al. 2010). The combination of HCV therapy and some types of ART regimen might specifically enhance the adverse event rates in HIV-1–HCV-coinfected women compared to HIV-1–HCV-coinfected men (Bhattacharya et al. 2010). Altogether, these data suggest that sex differences in disease progression in HIV-1–HCV coinfection require further investigation.

5.3.4.2 Tuberculosis Coinfection

Tuberculosis (TB) is an important cause of mortality and morbidity in HIV-1-infected individuals in Africa (Habib 2009; Small 2009; Brinkhof et al. 2007). In 2008, TB contributed to 26 % of the estimated deaths due to HIV-1 infection

(2009). According to UNAIDS 2013 Report, in 2012, people living with HIV-1 accounted for 1.1 million (13 %) of the estimated 8.7 million people globally who developed TB (2013). Individuals with latent TB are more likely to reactivate the infection and to experience rapidly progressive TB, including the selection of multidrug-resistant (MDR) TB when coinfected with HIV-1. HIV-1-infected patients are more susceptible to TB infection and mortality due to their compromised immune systems. It is generally accepted that in countries with a high prevalence of HIV-1, more women than men are diagnosed with TB (Getahun et al. 2010), which contrasts with the preponderance of adult men with TB being detected globally (reviewed in Chap. 8). In addition, in the settings of high HIV-1 prevalence, young women aged 15–24 experience TB rates 1.5–2 times higher than men in the same age group (Deluca et al. 2009). HIV-1-infected pregnant women appeared particularly at risk (Kali et al. 2006; Pillay et al. 2004; Ahmed et al. 1999). In contrast, Fenner and colleagues reported an association between male sex and a higher risk of TB in HIV-1-infected individuals (Fenner et al. 2011). However, they attributed this contrasting result to potential sex differences in ART utilization, with more women using ART. Indeed, antiretroviral therapy reduces by 65 % the risk that a person living with HIV-1 will develop TB and HIV-1 treatment lowers the risk of death among people living with HIV-1 who have TB by about 50 % (2013). Altogether, these data support a link between sex, HIV-1 infection, and TB. Given the major health threat posed by HIV-1 and TB infections, this link requires further investigation.

5.4 Sex Hormones and the Natural Course of HIV-1 Infection

Sex-specific differences can be attributable to the difference in anatomy, to X or Y chromosome-linked factors such as X chromosome inactivation or regulatory genes encoded on the Y chromosome, or to the effect of sex hormones. Although much of our focus will be on sex hormones, to emphasize the effects of sex chromosome-liked genes, Siddiqui and colleagues determined an association between an X chromosomal single-nucleotide polymorphism (SNP) and HIV-1 disease progression in women, but not in men (Siddiqui et al. 2009).

5.4.1 Changes in Sex Hormone Levels During HIV-1 Infection

5.4.1.1 Effects of the Menstrual Cycle in Women

CD4+ T cell count, HIV-1 RNA levels, CD4 expression, and CCR5 expression have been shown to fluctuate during the menstrual cycle in HIV-1-infected women

(Greenblatt et al. 2000; Reichelderfer et al. 2000; Yeaman et al. 2003). Several studies suggest that plasma HIV-1 load and HIV-1 shedding in the lower reproductive tract are lowest during the follicular stage and increase during the luteal stage (i.e., when progesterone levels are high) (Al-Harthi et al. 2001; Benki et al. 2004; Hanna 1999; Money et al. 2003). The menstrual cycle itself might be associated with significant variation in mucosal immunity and HIV-1 susceptibility. Indeed, macaques are more susceptible to SIV vaginal challenge during the luteal (i.e., when progesterone is dominant) phase of the menstrual cycle (Vishwanathan et al. 2011) compared to the follicular phase (i.e., when estradiol is dominant) (Sodora et al. 1998). This might be related to sex hormone-mediated alteration of the thickness of the mucosa epithelium and will be further discussed below.

5.4.1.2 Pregnancy

It is generally acknowledged that pregnancy does not accelerate HIV-1 disease progression (1997, Alliegro et al. 1997; Bessinger et al. 1998; Brettle et al. 1995; Saada et al. 2000; Selwyn et al. 1989; Weisser et al. 1998; Prins et al. 2005). Pregnancy also does not affect CD4+ T cell counts (van Benthem et al. 2002). However, increased risk of HIV-1 seroconversion has been reported among both antenatal and postnatal women (i.e., stages of pregnancy characterized by increased progesterone production) and appeared to be linked to pregnancy-associated disturbance of the vaginal flora (Taha et al. 1998; Quinn and Overbaugh 2005; Gray et al. 2005).

5.4.1.3 Menopause

CD4+ T cell count including those following ART initiation is similar between pre- and postmenopausal women (van Benthem et al. 2002; Patterson et al. 2009). Similarly, no significant differences in HIV-1 RNA levels after ART initiation are observed between pre- and postmenopausal women (Patterson et al. 2009). However, postmenopausal HIV-1-infected women have increased risk factors for metabolic complications, including osteoporosis, as well as lipid and glucose disturbances resulting from the impact of HIV-1 itself, ART, or due to the loss of the protective effects of estrogens. More studies are needed to investigate the impact of the natural changes in sex hormone levels associated with menopause on HIV-1 infection given the aging of the HIV-1-infected population.

5.4.1.4 Effect of HIV-1 on Sex Hormone Levels

HIV-1/AIDS has been associated with gonadal dysfunction (Minkoff et al. 1999; Kirkham and Lobb 1998; Chirgwin et al. 1996). Early menopause has been observed in HIV-1-infected women (Cejtin et al. 2006; de Pommerol et al. 2011;

Schoenbaum et al. 2005; Boonyanurak et al. 2012; Ferreira et al. 2007) with a greater degree of symptoms and with a different reproductive hormone profile than HIV-1-negative women (Ferreira et al. 2007; Miller et al. 2005b; Yin et al. 2012). Advanced stage of HIV-1 disease was the main predictor of early menopause (Csajka et al. 2004). Some menopausal characteristics are similar to symptoms of HIV-1 infection or to side effects of HIV-1 medication, such as menstrual cycle irregularities, skin and hair changes, emotional changes, or night sweats, and might therefore be difficult to distinguish. Furthermore, children infected with HIV-1 perinatally demonstrated significant delays in pubertal onset, particularly among those with more advanced HIV-1 disease (de Martino et al. 2001; Gertner et al. 1994; Mahoney et al. 1999; Majaliwa et al. 2009; Ratner Kaufman et al. 1997; Stagi et al. 2010; Williams et al. 2013). While ART appeared to reduce those delays, the potency of this effect might be different between boys and girls (Anderson et al. 2003; Herman-Giddens et al. 1997, 2001; Karpati et al. 2002; Sorensen et al. 2012; Buchacz et al. 2003). HIV-1 infection might directly or indirectly—through cytokine-induced inhibition of gonadotropin secretion (Zeitler et al. 1999)—affect production or secretion of hormones that regulate or control pubertal initiation and the pace of pubertal maturation (Buchacz et al. 2003). Delayed pubertal development in HIV-1-infected children has furthermore been attributed in part to reduced adrenal androgen secretion (Chantry et al. 2007; Ratner Kaufman et al. 1997). Androgen deficiency is common among HIV-1-infected women (Dolan et al. 2004; Grinspoon et al. 1997, 2001; Miller et al. 1998) and associated with reduced lean body mass, functional status, and bone density. Research on sex-specific treatment strategies for HIV-1-infected women has been very limited. Some studies have tested the impact of testosterone treatment in HIV-1-infected women to improve their quality of life indices (Choi et al. 2005; Dolan et al. 2004; Miller et al. 1998; Dolan Looby et al. 2009). In HIV-1-infected men, treatment of hypogonadism is routine and improves body composition, bone, and depression (Grinspoon et al. 1998a, b). Altogether, these data suggest that HIV-1 infection affects gonadal function, which can in turn influence HIV-1 pathogenesis as discussed below.

5.4.2 Role of Sex Hormones in HIV-1 Pathogenesis

5.4.2.1 Effect of Sex Hormones on Cytokine Production

Several studies have assessed the consequences of sex hormones on cytokine production. Ex vivo treatment with E2 and progesterone inhibits the production of Th1/inflammatory cytokines including interleukin (IL)-2, IFNγ, IL-12, IL-1β, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion from PBMCs from both HIV-1-uninfected and HIV-1-infected individuals, while no effect was observed on Th2/anti-inflammatory cytokines (Enomoto et al. 2007). Interestingly, E2- and progesterone-mediated inhibition of cytokine production was

greater in HIV-1-infected subjects (35 % decrease for both hormones) compared with uninfected subjects (12 % and 19 % for estrogen and progesterone, respectively) whereas the effect on proliferation and PBMC phenotype did not differ by HIV-1 status (Enomoto et al. 2007). IFNα production is also influenced by sex hormones (Seillet et al. 2012). Seillet and colleagues have dissected the mechanisms underlying hormonal regulation of IFNα production (Seillet et al. 2012). They showed that short-term in vitro exposure to 17β-estradiol (E2) did not affect the TLR-mediated responses of pDCs whereas in vivo treatment in postmenopausal women decreased the threshold of TLR responsiveness in human pDCs (Seillet et al. 2012), suggesting that the effect of E2 is independent of the presence of E2 at the time of TLR triggering in vitro but has an effect in vivo. Using a conditional mouse model, Seillet and colleagues further showed that the pDC IFNα response to TLR7 is positively regulated by E2 through estrogen receptor alpha (ERα) during pDC lineage differentiation from progenitors (Seillet et al. 2012). The impact of E2 on IFNα production was pDC specific, as no difference in cytokine production by monocytes was observed (Seillet et al. 2012). Altogether, these data suggest that the mechanisms underlying sex hormone regulation of cytokine production are very complex and precise, as both timing and cell specificity matter.

5.4.3 Hormonal Contraception and HIV-1 Disease Progression

For HIV-1-infected women, hormonal contraception prevents unintended pregnancy. For HIV-1-uninfected women, hormonal contraception should not increase the risk of HIV-1 acquisition. Different contraception methods exist, including oral contraceptives (OCs), contraceptive injections, and intrauterine devices (IUDs). The use of contraception (sterilization, hormonal, or intrauterine contraception) among HIV-1-infected women ranges from 4 to 30 % in France and the USA (Heard et al. 2004; Massad et al. 2007) and about 70 % among postpartum African women (Balkus et al. 2007). Increased use of hormonal contraception (up to 70 %) has been observed among HIV-1-discordant couples (Heard et al. 2004). IUDs are used at high rates globally but the proportion of women using IUDs in sub-Saharan Africa is small.

While a variety of data is available on the impact of hormonal contraception on HIV-1 acquisition, which will be discussed further below, studies on the impact of sex hormones on disease progression remain limited. The use of hormonal contraceptives has been associated with more rapid disease progression characterized by accelerated loss of CD4+ T cells and increased death rate in HIV-1-infected women (Stringer et al. 2007; Lavreys et al. 2006; Stringer and Antonsen 2008; Baeten et al. 2007b). Specifically, the use of the injectable contraceptive depot medroxyprogesterone acetate (DMPA; Depo-Provera) at the time of HIV-1 acquisition in Kenyan women was associated with higher viral load set points, a marker

for more rapid disease progression (Baeten et al. 2005). Although this association was not confirmed in other studies (Cejtin et al. 2003; Richardson et al. 2007; Stringer et al. 2009), it still raised the question of the safety of the use of hormonal contraception in HIV-1-infected women. Hormonal contraception might accelerate HIV-1-related disease progression by interfering with the body's natural immune responses. Progesterone and its derivatives have a broad immunosuppressive role. However, due to the physiological role of progesterone in the regulation of menstrual cycle and immune responsiveness in the female genital tract, progesterone functions are likely more pronounced in the genital tract tissue compared with the systemic compartment. Importantly, in contrast to progesterone, DMPA can also exert its biological activity through the glucocorticoid receptor that is widely expressed by various cells of the immune system (Kontula et al. 1983; Koubovec et al. 2005). Therefore, DMPA might exert stronger immunoregulatory functions than progesterone (Hel et al. 2010).

In particular, progesterone-based contraceptives might have an important role on the detrimental immune activation in HIV-1 infection. HIV-1-mediated impairment of mucosal barrier function associated with increased absorption of environmental microbial antigens to the systemic compartment is a major contributor to the deleterious chronic immune activation observed in HIV-1 infection (Brenchley et al. 2006). IgA plays a critical role in the regulation of the immune response to microbial community in the gut and in the reduction of inflammation induced by bacterial products and proinflammatory agents (Fernandez et al. 2003; Macpherson and Uhr 2004a, b). Progesterone and its derivatives mediate a strong effect on humoral immune responses in the genital tract and other mucosal tissues including the inhibition of IgG and IgA production and transepithelial transport (Franklin and Kutteh 1999; Kutteh et al. 1996; Lu et al. 1999, 2002, 2003; Nardelli-Haefliger et al. 2003; Patton et al. 2000; Shrier et al. 2003), decreased frequency of antibody-secreting cells in women and female macaques (Lu et al. 2002, 2003), decreased specific IgG and IgA responses after mucosal immunization with attenuated HSV-2, induction of permissive conditions for intravaginal infection of mice with HSV-2, and chlamydia trachomatis (Kaushic et al. 1998, 2003; Gillgrass et al. 2003). Therefore, the administration of progesterone-based contraceptives to HIV-1-infected women might further suppress antigen-specific IgA responses in mucosal tissues, causing more detrimental microbial translocation across the mucosal barrier in the chronic phase of infection.

Globally, recent studies and reviews found that the use of contraceptive methods is safe for HIV-1-infected women (Curtis et al. 2009; Stringer and Antonsen 2008; Phillips et al. 2013; Heffron et al. 2012, 2013; Hubacher et al. 2013). WHO recommend the use of any hormonal contraceptive method for women living with HIV-1. The relative efficacy and safety between the different contraceptive methods have only been poorly studied. Stringer and colleagues compared the efficacy and safety of hormonal contraceptives (DMPA or oral contraceptives) versus Cu-releasing intrauterine device (Cu-IUD) among 600 treatment-naïve HIV-1-infected Zairean women (Stringer et al. 2007). For unclear reasons, they found that women randomized to hormonal contraception were at increased risks of

HIV-1 progression and severe immunodeficiency or death compared to women using Cu-IUD (Stringer et al. 2007). Nevertheless, a further study showed that hormonal contraception was not associated with HIV-1 disease progression (Stringer et al. 2009). Taken together, these data highlight that hormonal contraceptive products and ART regimens might interact in unknown ways and hasten disease progression. Concomitant use should be carefully monitored.

5.5 Effect of Sex Hormones on HIV-1 Acquisition

5.5.1 Hormonal Contraception and HIV-1 Acquisition

The impact of contraception on the risk of HIV-1 acquisition has been extensively studied, mainly in developing countries. Epidemiological data obtained so far are inconclusive. Some studies have shown a correlation between the use of hormonal contraception and increased risk of HIV-1 infection (Guimaraes et al. 1995; Lavreys et al. 2004b; Leclerc et al. 2008; Martin et al. 1998a; Plourde et al. 1992; Plummer et al. 1991; Rehle et al. 1992; Sinei et al. 1996; Ungchusak et al. 1996; Wang et al. 1999; Watson-Jones et al. 2009; Baeten et al. 2007a). In particular, some studies have suggested an increased risk of HIV-1 acquisition linked to the use of the DMPA. This highly effective injectable progesterone-based contraceptive is used by more than 90 million women worldwide, and particularly common in sub-Saharan Africa, as it is only required to be administered every three months. A number of studies have found no increased risk of HIV-1 acquisition associated with the use of DMPA (Bulterys et al. 1994; Kapiga et al. 1998; Kiddugavu et al. 2003; Mati et al. 1995; Myer et al. 2007; Taneepanichskul et al. 1997). However, it has been shown in a 10-year prospective study involving more than 1,500 sex workers in Mombasa, Kenya, that women with DMPA had a twice-higher risk of acquiring HIV-1 than women without DMPA (Baeten et al. 2005; Lavreys et al. 2004a, b). Leclerc and colleagues observed similar findings in young African women and estimated that 6 % of new HIV-1 cases are attributable to DMPA use (Leclerc et al. 2008). The increased risk was specifically linked to the use of DMPA, as the use of OCs did not significantly increase the risk of HIV-1 seropositivity (Leclerc et al. 2008). Yet, it was shown elsewhere that the use of OCs was a risk factor of HIV-1 acquisition among high-risk women (Plummer et al. 1991) but not among women at low risk (Bulterys et al. 2007). The influence of the use of hormonal contraceptives on the risk of HIV-1 acquisition might differ according to the population studied, with an associated increased risk demonstrated only among high risk population and young women, even after controlling for confounding factors (such as demographic, exposure, or biologic) (Martin et al. 1998b; Baeten et al. 2007a). The conclusions of the recent report of McCoy and colleagues summarize well the actual “consensus” in regard to hormonal contraception and HIV-1 acquisition: “while oral contraceptives do not seem

to be associated with increased risk of HIV-1 acquisition, substantial uncertainty regarding the effect of many injectable hormonal contraception persists" (McCoy et al. 2013).

The elevated risks associated with the use of DMPA reported in multiple studies (Heffron et al. 2012; Mor et al. 2003; Morrison et al. 2010, 2012; Blish and Baeten 2011; Gray 2012; Morrison and Nanda 2012) have been questioned by potential methodological limitations and confounding behavioral factors (risk observed only in high-risk population). For example, unsafe sharing and reuse of needles and syringes used for delivering DMPA have been highlighted as a potential confounding factor (Gisselquist 2008). Indeed, a correlation between DMPA use and increased HCV infection rate has been demonstrated in Tanzania (Stark et al. 2000). The complex balance of risks and benefits of reducing the availability of effective contraceptive options for women has to be carefully considered, as it might have dramatic consequences on unintended pregnancies, which will result in an increase in maternal mortality related to pregnancy, child birth, or within 42 days of termination of pregnancy (Butler et al. 2013). Unintended pregnancies may also impact HIV-1 risk, even if the impact of pregnancy on HIV-1 acquisition remains conflicting (Gray et al. 2005; Morrison et al. 2007b; Mugo et al. 2011; Reid et al. 2010), and may increase levels of perinatal HIV-1 transmission among HIV-1-infected women. Therefore, reducing DMPA use might either decrease or increase the overall number of deaths depending on HIV-1 prevalence, birth rate, and the maternal mortality ratio, in addition to the true effect size of DMPA as exemplified in Fig. 5.3 (Butler et al. 2013). In countries with high HIV-1 prevalence and high use of DMPA, such as South Africa, reducing DMPA use might however be beneficial (Butler et al. 2013). Given the inconclusive nature of the body of evidence and the analysis of risks and benefits to country programs, the WHO and the CDC recommended that no changes in policy should be currently made. Official recommendations continue to state no restrictions on the use of any hormonal contraceptive method for women living with HIV-1 or at high risk of HIV-1 infection. Nevertheless, they correctly pointed out that more research is needed and that women using progestin-only injectable contraception should be strongly advised to also always use condom (2012b).

5.5.2 Effects of Sex Hormones on the Female Reproductive Tract

5.5.2.1 Effect of Endogenous and Exogenous Sex Hormones on Characteristics of the Genital Mucosa

As a general rule, progesterone increases susceptibility whereas estrogens protect against viral STIs (e.g., HIV-1 and HSV-2) [reviewed in Kaushic et al. (2011)]. Estrogens and progesterone affect vulnerability to viral STIs by inducing structural changes in the genital mucosa (Michael and Esfahani 1997; Jacobson et al. 2000; Sonnex 1998). The effect of hormonal contraception, particularly DMPA, is also

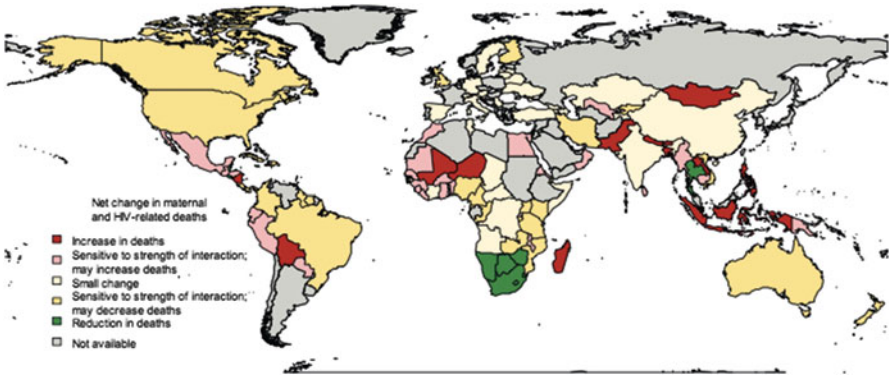

Fig. 5.3 The number of net maternal and HIV-1-related deaths resulting from cessation of injectable hormonal contraception (IHC) use is influenced by both HIV-1 prevalence and the use of IHC. Countries may therefore benefit or suffer from the cessation of IHC use. Direction of change. Countries are coloured according to the direction of change in the net number of maternal and HIV-related deaths that result directly from stopping IHC use assuming the relative risk equal to 1.2 (RR = 1.2) and RR = 2.19: *red*: expected increase in net maternal and HIV-related deaths (>0.5 % under both RR assumptions); *pink*: change in net deaths is dependent on the effect size (>0.5 % increase only when RR.1.2); *cream*: reductions in IHC use unlikely to provide public health benefit in terms of deaths prevented (<0.5 % change with both estimates); *yellow*: change in net deaths is dependent on the effect size (>0.5 % decrease only when RR = 2.19); *green*: expected decrease in net deaths (>0.5 % under both RR assumptions); *grey*: data not available

associated with an increased acquisition of cervical chlamydial, gonococcal infections, and candidiasis, which might increase the susceptibility to HIV-1 (Baeten et al. 2001; Lavreys et al. 2004c; Morrison et al. 2004). While high doses of progesterone can lead to thinning of the cervicovaginal epithelium and therefore enhance vaginal SIV acquisition by facilitating the access to target Langerhans cells, CD4+ T cells, and macrophages in the epithelium and subepithelial layers (Marx et al. 1996; Hild-Petito et al. 1998), estrogen induces thickening of the vaginal stratified squamous epithelium (Smith et al. 2000) and therefore might block access to target cells. Studies on macaques showed that this effect starts after 24 h of treatment and could last for at least one week (Smith et al. 2004). Most transmissions occur through damaged or atrophied vaginal epithelium which is more prone to trauma during sexual intercourse (Nilsson and Heimer 1992).(Hladik and McElrath 2008). Estrogen's protective role might also be mediated by the increase in cervical mucus production and decrease of cervical ectopy (Nicolosi et al. 1994; Brabin 2002; Jacobson et al. 2000; Myer et al. 2006). In contrast, oral contraceptives might increase cervical ectopy (Critchlow et al. 1995), which will increase HIV-1 susceptibility.

Furthermore, sex hormones might affect HIV-1 acquisition through the changes in the vaginal milieu and bacterial flora (Mingjia and Short 2002). Estradiol treatment decreases cervicovaginal pH in women and female macaques, making it hostile to the virus (Castelo-Branco et al. 2005; Smith 1993; Molander et al. 1990; Smith et al. 2000). It has been demonstrated that women without lactobacillus or

with abnormal vaginal microbiota have higher acquisition rates of HIV-1 compared to those with H_2O_2-producing lactobacillus (Klebanoff and Coombs 1991; Martin et al. 1999). Progesterone and DMPA treatment decreased colonization by H_2O_2-positive lactobacillus (Miller et al. 2000; Mingjia and Short 2002). The production of H_2O_2 by lactobacillus represents an important mechanism by which it maintains dominance over other vaginal microbiota. Colonization with lactobacillus might prevent bacterial vaginosis that is associated with increased risk of HIV-1 transmission (Martin et al. 1998a; Atashili et al. 2008; Taha et al. 1998).

Sex hormones can also influence the frequency of available target cells for HIV-1 infection. Exogenous estrogens decrease (Wira et al. 2010) whereas progesterone increases (Wieser et al. 2001) the frequency of LCs in the vaginal epithelial and stromal tissue. Peak estrogen levels decrease the recruitment of inflammatory T cells and macrophages through downregulation of intercellular adhesion molecule-1 (ICAM-1), E-selectins, and vascular cell adhesion molecule-1 (VCAM-1) (Straub 2007). The expression of HIV-1 receptors CD4, CCR5, and CXCR4 on human cervical CD4+ T cells is increased by progesterone (Dominguez et al. 2003; Sheffield et al. 2009; Carias et al. 2013; Prakash et al. 2002). Sex steroids can furthermore upregulate HIV-1 gene expression (Furth et al. 1990). Progesterone-based contraceptives also appear to increase the number of inflammatory cells in cervicovaginal fluid (Ghanem et al. 2005). Taken together, these data suggest that the vaginal epithelium undergoes major cyclical changes in its structure, which might determine the risk for HIV-1 acquisition. Further research on the efficacy of topical pre-exposure prophylaxis (PrEP) is required to carefully account for those changes.

5.5.2.2 Effect of Sex Hormones on Immune Responses in the Female Reproductive Tract

Sex hormones might also influence HIV-1 acquisition by their broad effect on the regulation of innate and adaptive immune defenses in the female reproductive tract [reviewed in Kaushic et al. (2010); Wira et al. 2010) (Beagley and Gockel 2003; Paavonen 1994)]. Progesterone causes several immunoregulatory effects, including inhibition of T cell responses and cytotoxic activity (Borel et al. 1999; Cherpes et al. 2008; Laskarin et al. 1999; Wyle and Kent 1977), decreased frequency of antibody-secreting cells in women and female macaques (Lu et al. 2002, 2003), and inhibition of pDC TLR9-induced IFNα production (Hughes et al. 2008), which might contribute to the increased shedding of HIV-1 in the genital tract of women using contraception (Clemetson et al. 1993; Mostad et al. 1997; Wang et al. 2004; Nag et al. 2004). Of note, CTL activity appeared to be distinctly regulated by sex hormones in the genital tract mucosa and peripheral blood (White et al. 2001; Wieser et al. 2001), which could be due to local inflammation or variations in viral replication in the systemic versus mucosal compartments (Hel et al. 2010). High estradiol levels decrease the frequency of LCs in vaginal epithelium (Wieser et al. 2001) and the recruitment of inflammatory T cells and macrophages through

downregulation of ICAM-1, VCAM-1, and E-selectins (Straub 2007; Harkonen and Vaananen 2006; Ito et al. 2002; Salem et al. 2000; Zang et al. 2002). Progesterone immunoregulatory function might be potentiated in HIV-1 infection as suggested by recent findings of Enomoto and colleagues on stronger inhibitory effects of progesterone on T cell proliferation and Th1-type cytokine production in HIV-1-infected individuals as compared to uninfected individuals (Enomoto et al. 2007). Huijbregts and colleagues recently demonstrated that MPA suppresses the production of IFN-γ, IL-2, IL-4, IL-6, IL-12, TNFα, and MIP-1α by peripheral blood cells and activated T cells and reduces the TLR7/9-mediated production of IFN**α** and TNF**α** by pDCs (Huijbregts et al. 2013). Dose–response analysis suggest that women would be particularly vulnerable to the immunosuppressive effects of medroxyprogesterone acetate in PBMCs shortly after injection and that susceptibility could vary greatly between individuals (Huijbregts et al. 2013). Overall, these data suggest that endogenous and/or exogenous sex hormones have pleiotropic effects on the mucosal immune environment. In the context of PrEP research, further studies are needed to characterize the potential effect of sex hormones on the expression of cellular transporters involved in the uptake of drugs.

5.5.2.3 Effect of Hormonal Contraception on Cervicovaginal Shedding of HIV-1

The circulating HIV-1 load is a major determinant of cervicovaginal shedding of HIV-1 RNA, even among women using antiretroviral medication (Benki et al. 2004; Kovacs et al. 2001). Viral shedding refers to the successful reproduction, expulsion, and host–cell infection caused by virus progeny. The use of hormonal contraception has been associated with increased cervicovaginal shedding of HIV-1, with a significant dose dependency on progesterone levels (Clemetson et al. 1993; Mostad et al. 1997; Wang et al. 2004), in which higher progesterone levels favor HIV-1 production (Morrison et al. 2007a, 2010) (Vishwanathan et al. 2011) or no increase in HIV-1 production at all (Kovacs et al. 2001). Similarly, the use of intrauterine devices (Cu-releasing intrauterine device/ Cu-IUDs or levonorgestrel intrauterine device/ LNG-IUDs) did not increase cervical shedding of HIV-1 (Heikinheimo et al. 2006; Richardson et al. 1999). Besides, it has been suggested that the use of hormonal contraception might increase the risk of acquiring multiple variants of HIV-1 among Kenyan sex workers (Sagar et al. 2004b). Given these somehow conflicting results, it seems crucial to further characterize the effect of specific contraceptive methods including the kinetics of the concentration of the exogenous sex hormones.

5.5.2.4 SIV Model of Transmission to Study Effect of Hormonal Contraception

The impact of hormonal contraceptives on HIV-1 acquisition was studied in the SIV model, allowing for more controlled settings. The administration of DMPA enhanced vaginal transmission of SIV by more than sevenfold (Trunova et al. 2006; Veazey et al. 2003b) (Marx et al. 1996). This effect might have been mediated by the thinning of the vaginal epithelium (Marx et al. 1996; Abel et al. 2004) (Hild-Petito et al. 1998; Smith et al. 2000) but also possibly by the alteration of immune responses (Trunova et al. 2006; Veazey et al. 2003b) (Genesca et al. 2007). Indeed, the late appearance (i.e., 2–3 weeks postinfection) of differences in plasma viremia in the DMPA-naïve and DMPA-treated infected macaques suggests an immunological rather than transmission-dependent mechanism (Trunova et al. 2006). Similarly to what has been observed in humans, progesterone-based hormone replacements have been reported to inhibit cytokine production in nonhuman primates (Attanasio et al. 2002; Keller et al. 2001; Lu et al. 2002). The immunosuppressive effect of DMPA might increase viral burden and facilitate the transmission of multiple variants as a result of the absence of selective immune pressures (Trunova et al. 2006). However, the increased susceptibility to SIV infection in response to DMPA pretreatment is likely to be driven by its local effects on the genital tract rather than to systemic effects. DMPA administration selectively abrogated the protective effect of an attenuated lentivirus-induced protection against intravaginal challenge with live-attenuated lentivirus (Abel et al. 2004; Genesca et al. 2007) but did not alter protection after intravenous SIV challenge in female macaques immunized with a live-attenuated lentivirus (Genesca et al. 2010). Altogether, these results suggest that hormonal contraceptives such as DMPA might alter immune responses differently according to the nature of SIV challenges, which might have crucial implication for vaccine efficacy. Effects of hormonal contraceptives on vaccine efficacy need to be considered while assessing potential candidates for an AIDS vaccine.

Important differences between human and macaques might lead to discrepant results between the two species. First the decrease in progesterone levels during the 3-monthly injection period might have different kinetics in blood and mucosal tissues among women and female macaques. Progesterone treatment has been historically used in SIV challenge models to increase transmission efficiency and standardize vaginal SIV infection (Veazey et al. 2003b) (Poonia et al. 2006). The higher doses of challenge virus generally used in primate studies might have biased the results as not properly recapitulating the biological effects observed in women. An appropriate model will require the identification of a physiologic DMPA dose that suppresses ovulation and mimics other biological effects seen in women (Radzio et al. 2014). The virus delivery medium differs between humans (semen) and macaques (culture medium). Last but not least, the effect of progesterone on the vaginal epithelium of humans might be less profound, with several studies actually reporting increased epithelial thickness caused by hyperplasia (Ildgruben

et al. 2003; Mauck et al. 1999; Miller et al. 2000) or no thinning at all in women during the use of DMPA (Bahamondes et al. 2000; Mauck et al. 1999) contrary to what observed in primates SIV model (Hild-Petito et al. 1998). Studies using larger cohorts of animals as well as more physiologically relevant models are still needed to fully elucidate the impact of DMPA on virus transmission. Radzio et al. recently highlighted the advantages of the pigtail macaque model to study DMPA effects on SIV transmission (Radzio et al. 2014). Pigtail macaques have normal menstrual cycles and fluctuations in sex hormone levels that are similar to women as opposed to rhesus macaques that have seasonal breeding (Mauck et al. 1999; Radzio et al. 2012; Sodora et al. 1998; Steiner et al. 1977). Optimal DMPA dose was determined to recapitulate in pigtail macaques the biological effect seen in women: (1) ovulation suppression and (2) modest reductions in vaginal epithelium thickness that are similar in magnitude to those seen in women. In this model, physiologic DMPA dose did not increase mucosal virus shedding (Radzio et al. 2014).

Altogether, these data highlight the great potential of SIV model to study the effect of hormonal contraception on HIV-1 transmission but also its limitations, related to its intrinsic nature, that have to be carefully accounted for.

5.6 Conclusion

Numerous epidemiological studies have documented differences between men and women in acquisition rates and manifestations of HIV-1 infection and have lead in some instances to controversial reports, notably due to underlying socioeconomic factors. One major and very consistent finding revealed by those studies is the lower viremia observed in HIV-1-infected women compared to HIV-1-infected men, in particular during early stages of infection. It is also generally acknowledged that women display a greater susceptibility to HIV-1 acquisition. To date, considerably fewer studies have addressed the mechanisms underlying those sex differences but have led to some interesting observations. The use of the SIV rhesus macaque model has been very helpful to elucidate the biological characteristics of the female genital tract responsible for the greater susceptibility of females to HIV-1 acquisition. Similarly, advances have recently been made in the understanding of how sex differences in primary innate immune responses can impact HIV-1 pathogenesis. Higher IFNα production by pDCs from women than in men in response to HIV-1 might at least partially account for the higher immune activation and subsequent faster disease progression observed in HIV-1-infected women for the same level of viral replication as men. Recent data have pointed toward an important role of sex hormones in mediating the sex differences observed in innate immune responses to HIV-1 infection. Nevertheless, the role of genes encoded by the X chromosome, such as *TLR7*, *TLR8,* and *FoxP3*, should be further investigated. Additional efforts are needed to better dissect the molecular mechanisms responsible for observed differences in the manifestations of HIV-1 disease between women and men. The consequences of the well-described sex differences in HIV-1 viral loads on the

establishment and maintenance of the viral reservoir have yet to be described, a topic highly relevant to the ongoing research on HIV-1 cure. More large-scale studies and clinical trials rigorously considering sex in the experimental design and analysis should provide basis for strategic design of individualized treatment and prevention methods.

References

(1981) Pneumocystis pneumonia – Los Angeles. MMWR Morb Mortal Wkly Rep 30:250–252
(1992) Comparison of female to male and male to female transmission of HIV in 563 stable couples European Study Group on Heterosexual Transmission of HIV. BMJ 304:809–813
(1994) Immunologic marker paths for seroconversion: single determinations of immunoglobulin A and beta 2-microglobulin are not adequate to estimate time of HIV infection. Multicohort Analysis Project Workshop. Part II. AIDS 8: 923–933
(1997) Immunological markers in HIV-infected pregnant women. The European Collaborative Study and the Swiss HIV Pregnancy Cohort. AIDS 11: 1859–1865
(1999) The mode of delivery and the risk of vertical transmission of human immunodeficiency virus type 1--a meta-analysis of 15 prospective cohort studies. The International Perinatal HIV Group. N Engl J Med 340: 977–987
(2000) Time from HIV-1 seroconversion to AIDS and death before widespread use of highly-active antiretroviral therapy: a collaborative re-analysis. Collaborative Group on AIDS Incubation and HIV Survival including the CASCADE EU Concerted Action. Concerted Action on SeroConversion to AIDS and Death in Europe. Lancet 355:1131–1137
(2001) First report of AIDS. MMWR Morb Mortal Wkly Rep 50:429
(2002) Level and pattern of HIV-1-RNA viral load over age: differences between girls and boys? AIDS 16:97–104
(2003) Differences in CD4 cell counts at seroconversion and decline among 5739 HIV-1-infected individuals with well-estimated dates of seroconversion. J Acquir Immune Defic Syndr, 34: 76–83
(2006) Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1-infected adults in Africa. AIDS 20:1391–1399
(2008) Report on the global AIDS epidemic. UNAIDS
(2010a) Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996-2006: collaborative analysis of 13 HIV cohort studies. Clin Infect Dis 50:1387–1396
(2010b) Seroprevalence of herpes simplex virus type 2 among persons aged 14-49 years--United States, 2005–2008. MMWR Morb Mortal Wkly Rep 59:456–459
(2012a) Global Report on the HIV Epidemic (Geneva: UNAIDS). UNAIDS
(2012b) Hormonal contraception and HIV: Technical statement. WHO
(2012c) Update to CDC's U.S. Medical Eligibility Criteria for Contraceptive Use, 2010: revised recommendations for the use of hormonal contraception among women at high risk for HIV infection or infected with HIV. MMWR Morb Mortal Wkly Rep 61:449–452
(2013) Global report: UNAIDS report on the global AIDS epidemic 2013. UNAIDS
Abel K, Rourke T, Lu D, Bost K, Mcchesney MB, Miller CJ (2004) Abrogation of attenuated lentivirus-induced protection in rhesus macaques by administration of depo-provera before intravaginal challenge with simian immunodeficiency virus mac239. J Infect Dis 190: 1697–1705
Adjorlolo-Johnson G, De Cock KM, Ekpini E, Vetter KM, Sibailly T, Brattegaard K, Yavo D, Doorly R, Whitaker JP, Kestens L et al (1994) Prospective comparison of mother-to-child transmission of HIV-1 and HIV-2 in Abidjan, Ivory Coast. JAMA 272:462–466
Ahdieh-Grant L, Li R, Levine AM, Massad LS, Strickler HD, Minkoff H, Moxley M, Palefsky J, Sacks H, Burk RD, Gange SJ (2004) Highly active antiretroviral therapy and cervical

squamous intraepithelial lesions in human immunodeficiency virus-positive women. J Natl Cancer Inst 96:1070–1076

Ahmed Y, Mwaba P, Chintu C, Grange JM, Ustianowski A, Zumla A (1999) A study of maternal mortality at the University Teaching Hospital, Lusaka, Zambia: the emergence of tuberculosis as a major non-obstetric cause of maternal death. Int J Tuberc Lung Dis 3:675–680

Al-Harthi L, Kovacs A, Coombs RW, Reichelderfer PS, Wright DJ, Cohen MH, Cohn J, Cu-Uvin S, Watts H, Lewis S, Beckner S, Landay A (2001) A menstrual cycle pattern for cytokine levels exists in HIV-positive women: implication for HIV vaginal and plasma shedding. AIDS 15: 1535–1543

Alabi AS, Jaffar S, Ariyoshi K, Blanchard T, Schim Van Der Loeff M, Awasana AA, Corrah T, Sabally S, Sarge-Njie R, Cham-Jallow F, Jaye A, Berry N, Whittle H (2003) Plasma viral load, Cd4 cell percentage, Hla and survival of HIV-1, HIV-2, and dually infected Gambian patients. AIDS 17:1513–1520

Allain JP (1986) Prevalence of HTLV-III/LAV antibodies in patients with hemophilia and in their sexual partners in France. N Engl J Med 315:517–518

Alliegro MB, Dorrucci M, Phillips AN, Pezzotti P, Boros S, Zaccarelli M, Pristera R, Rezza G (1997) Incidence and consequences of pregnancy in women with known duration of HIV infection. Italian Seroconversion Study Group. Arch Intern Med 157:2585–2590

Alric L, Fort M, Izopet J, Vinel JP, Bureau C, Sandre K, Charlet JP, Beraud M, Abbal M, Duffaut M (2000) Study of host- and virus-related factors associated with spontaneous hepatitis C virus clearance. Tissue Antigens 56:154–158

Althoff KN, Rebeiro P, Brooks JT, Buchacz K, Gebo K, Martin J, Hogg R, Thorne JE, Klein M, Gill MJ, Sterling TR, Yehia B, Silverberg MJ, Crane H, Justice AC, Gange SJ, Moore R, Kitahata MM, Horberg MA (2014) Disparities in the quality of HIV care when using US Department of Health and Human Services indicators. Clin Infect Dis 58:1185–1189

Amaro H (1995) Love, sex, and power. Considering women's realities in HIV prevention. Am Psychol 50:437–447

Anastos K, Gange SJ, Lau B, Weiser B, Detels R, Giorgi JV, Margolick JB, Cohen M, Phair J, Melnick S, Rinaldo CR, Kovacs A, Levine A, Landesman S, Young M, Munoz A, Greenblatt RM (2000) Association of race and gender with HIV-1 Rna levels and immunologic progression. J Acquir Immune Defic Syndr 24:218–226

Anderson SE, Dallal GE, Must A (2003) Relative weight and race influence average age at menarche: results from two nationally representative surveys of US girls studied 25 years apart. Pediatrics 111:844–850

Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS (2008) Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. AIDS 22:1493–1501

Athreya BH, Pletcher J, Zulian F, Weiner DB, Williams WV (1993) Subset-specific effects of sex hormones and pituitary gonadotropins on human lymphocyte proliferation in vitro. Clin Immunol Immunopathol 66:201–211

Attanasio R, Gust DA, Wilson ME, Meeker T, Gordon TP (2002) Immunomodulatory effects of estrogen and progesterone replacement in a nonhuman primate model. J Clin Immunol 22: 263–269

Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta R, Puren A (2005) Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. PLoS Med 2:e298

Baeten JM, Benki S, Chohan V, Lavreys L, Mcclelland RS, Mandaliya K, Ndinya-Achola JO, Jaoko W, Overbaugh J (2007a) Hormonal contraceptive use, herpes simplex virus infection, and risk of HIV-1 acquisition among Kenyan women. AIDS 21:1771–1777

Baeten JM, Lavreys L, Overbaugh J (2007b) The influence of hormonal contraceptive use on HIV-1 transmission and disease progression. Clin Infect Dis 45:360–369

Baeten JM, Lavreys L, Sagar M, Kreiss JK, Richardson BA, Chohan B, Panteleeff D, Mandaliya K, Ndinya-Achola JO, Overbaugh J, Farley T, Mwachari C, Cohen C, Chipato T, Jaisamrarn U, Kiriwat O, Duerr A (2005) Effect of contraceptive methods on natural history of HIV: studies from the Mombasa cohort. J Acquir Immune Defic Syndr 38(Suppl 1):S18–S21

Baeten JM, Nyange PM, Richardson BA, Lavreys L, Chohan B, Martin HL Jr, Mandaliya K, Ndinya-Achola JO, Bwayo JJ, Kreiss JK (2001) Hormonal contraception and risk of sexually transmitted disease acquisition: results from a prospective study. Am J Obstet Gynecol 185:380–385

Bahamondes L, Trevisan M, Andrade L, Marchi NM, Castro S, Diaz J, Faundes A (2000) The effect upon the human vaginal histology of the long-term use of the injectable contraceptive Depo-Provera. Contraception 62:23–27

Baker JV, Duprez D (2010) Biomarkers and HIV-associated cardiovascular disease. Curr Opin HIV AIDS 5:511–516

Balkus J, Bosire R, John-Stewart G, Mbori-Ngacha D, Schiff MA, Wamalwa D, Gichuhi C, Obimbo E, Wariua G, Farquhar C (2007) High uptake of postpartum hormonal contraception among HIV-1-seropositive women in Kenya. Sex Transm Dis 34:25–29

Ballesteros-Zebadua P, Villarreal C, Cocho G, Huerta L, Estrada JL (2013) Differences in HIV-1 viral loads between male and female antiretroviral-untreated Mexican patients. Arch Med Res 44:296–301

Barber TJ, Geretti AM, Anderson J, Schwenk A, Phillips AN, Bansi L, Gilson R, Hill T, Walsh J, Fisher M, Johnson M, Post F, Easterbrook P, Gazzard B, Palfreeman A, Orkin C, Leen C, Gompels M, Dunn D, Delpech V, Pillay D, Sabin CA (2011) Outcomes in the first year after initiation of first-line Haart among heterosexual men and women in the UK Chic Study. Antivir Ther 16:805–814

Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (Aids). Science 220:868–871

Beagley KW, Gockel CM (2003) Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. FEMS Immunol Med Microbiol 38:13–22

Beau JP, Imboua-Coulibaly L (1999) HIV-related gender biases among malnourished children in Abidjan, Cote D'Ivoire. J Trop Pediatr 45:169–171

Begaud E, Chartier L, Marechal V, Ipero J, Leal J, Versmisse P, Breton G, Fontanet A, Capoulade-Metay C, Fleury H, Barre-Sinoussi F, Scott-Algara D, Pancino G (2006) Reduced Cd4 T cell activation and in vitro susceptibility to HIV-1 infection in exposed uninfected Central Africans. Retrovirology 3:35

Beignon AS, Mckenna K, Skoberne M, Manches O, Dasilva I, Kavanagh DG, Larsson M, Gorelick RJ, Lifson JD, Bhardwaj N (2005) Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor-viral Rna interactions. J Clin Invest 115:3265–3275

Belec L, Dupre T, Prazuck T, Tevi-Benissan C, Kanga JM, Pathey O, Lu XS, Pillot J (1995) Cervicovaginal overproduction of specific IgG to human immunodeficiency virus (HIV) contrasts with normal or impaired IgA local response in HIV infection. J Infect Dis 172: 691–697

Benedetti P, Greco D, Figoli F, Tirelli U (1991) Epidemic Kaposi's sarcoma in female Aids patients–a report of 23 Italian cases. AIDS 5:466–467

Benhamou Y, Bochet M, Di Martino V, Charlotte F, Azria F, Coutellier A, Vidaud M, Bricaire F, Opolon P, Katlama C, Poynard T (1999) Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. Hepatology 30:1054–1058

Benki S, Mostad SB, Richardson BA, Mandaliya K, Kreiss JK, Overbaugh J (2004) Cyclic shedding of HIV-1 Rna in cervical secretions during the menstrual cycle. J Infect Dis 189:2192–2201

Berghofer B, Frommer T, Haley G, Fink L, Bein G, Hackstein H (2006) Tlr7 ligands induce higher Ifn-alpha production in females. J Immunol 177:2088–2096

Bessinger R, Clark R, Kissinger P, Rice J, Coughlin S (1998) Pregnancy is not associated with the progression of HIV disease in women attending an HIV outpatient program. Am J Epidemiol 147:434–440

Bhattacharya D, Umbleja T, Carrat F, Chung RT, Peters MG, Torriani F, Andersen J, Currier JS (2010) Women experience higher rates of adverse events during hepatitis C virus therapy in HIV infection: a meta-analysis. J Acquir Immune Defic Syndr 55:170–175

Biancotto A, Grivel JC, Iglehart SJ, Vanpouille C, Lisco A, Sieg SF, Debernardo R, Garate K, Rodriguez B, Margolis LB, Lederman MM (2007) Abnormal activation and cytokine spectra in lymph nodes of people chronically infected with HIV-1. Blood 109:4272–4279

Biasin M, Caputo SL, Speciale L, Colombo F, Racioppi L, Zagliani A, Ble C, Vichi F, Cianferoni L, Masci AM, Villa ML, Ferrante P, Mazzotta F, Clerici M (2000) Mucosal and systemic immune activation is present in human immunodeficiency virus-exposed seronegative women. J Infect Dis 182:1365–1374

Bica I, Mcgovern B, Dhar R, Stone D, Mcgowan K, Scheib R, Snydman DR (2001) Increasing mortality due to end-stage liver disease in patients with human immunodeficiency virus infection. Clin Infect Dis 32:492–497

Biggar RJ, Chaturvedi AK, Goedert JJ, Engels EA (2007) Aids-related cancer and severity of immunosuppression in persons with Aids. J Natl Cancer Inst 99:962–972

Biggar RJ, Taha TE, Hoover DR, Yellin F, Kumwenda N, Broadhead R (2006) Higher in utero and perinatal HIV infection risk in girls than boys. J Acquir Immune Defic Syndr 41:509–513

Biggar RJ, Wohlfahrt J, Westergaard T, Melbye M (1999) Sex ratios, family size, and birth order. Am J Epidemiol 150:957–962

Bjorck P (2004) Dendritic cells exposed to herpes simplex virus in vivo do not produce IFN-alpha after rechallenge with virus in vitro and exhibit decreased T cell alloreactivity. J Immunol 172: 5396–5404

Blacker J (2004) The impact of Aids on adult mortality: evidence from national and regional statistics. AIDS 18(Suppl 2):S19–S26

Blaskewicz CD, Pudney J, Anderson DJ (2011) Structure and function of intercellular junctions in human cervical and vaginal mucosal epithelia. Biol Reprod 85:97–104

Blish CA, Baeten JM (2011) Hormonal contraception and HIV-1 transmission. Am J Reprod Immunol 65:302–307

Boasso A, Hardy AW, Anderson SA, Dolan MJ, Shearer GM (2008) HIV-induced type I interferon and tryptophan catabolism drive T cell dysfunction despite phenotypic activation. PLoS One 3: e2961

Boasso A, Shearer GM (2008) Chronic innate immune activation as a cause of HIV-1 immunopathogenesis. Clin Immunol 126:235–242

Bohlius J, Valeri F, Maskew M, Prozesky H, Garone D, Sengayi M, Fox MP, Davies MA, Egger M (2014) Kaposi's Sarcoma in HIV-infected patients in South Africa: Multicohort study in the antiretroviral therapy era. Int J Cancer 135:2644–2652

Boily MC, Baggaley RF, Wang L, Masse B, White RG, Hayes RJ, Alary M (2009) Heterosexual risk of HIV-1 infection per sexual act: systematic review and meta-analysis of observational studies. Lancet Infect Dis 9:118–129

Bolan G, Ehrhardt A, Wasserheit JN (1999) Gender perspectives and STDs. In: Holmes KK, Sparling P, Mardh PA et al (eds) Sexually transmitted diseases. McGraw-Hill, New York, NY

Bonacini M, Louie S, Bzowej N, Wohl AR (2004) Survival in patients with HIV infection and viral hepatitis B or C: A cohort study. AIDS 18:2039–2045

Bonnet F, Lewden C, May T, Heripret L, Jougla E, Bevilacqua S, Costagliola D, Salmon D, Chene G, Morlat P (2004) Malignancy-related causes of death in human immunodeficiency virus-infected patients in the era of highly active antiretroviral therapy. Cancer 101:317–324

Boonyanurak P, Bunupuradah T, Wilawan K, Lueanyod A, Thongpaeng P, Chatvong D, Sophonphan J, Saeloo S, Ananworanich J, Chaithongwongwatthana S (2012) Age at menopause and menopause-related symptoms in human immunodeficiency virus-infected Thai women. Menopause 19:820–824

Borel IM, Freire SM, Rivera E, Canellada A, Binaghi RA, Margni RA (1999) Modulation of the immune response by progesterone-induced lymphocyte factors. Scand J Immunol 49:244–250

Bosch RJ, Bennett K, Collier AC, Zackin R, Benson CA (2007) Pretreatment factors associated with 3-year (144-week) virologic and immunologic responses to potent antiretroviral therapy. J Acquir Immune Defic Syndr 44:268–277

Bosinger SE, Hosiawa KA, Cameron MJ, Persad D, Ran L, Xu L, Boulassel MR, Parenteau M, Fournier J, Rud EW, Kelvin DJ (2004) Gene expression profiling of host response in models of acute HIV infection. J Immunol 173:6858–6863

Bosinger SE, Li Q, Gordon SN, Klatt NR, Duan L, Xu L, Francella N, Sidahmed A, Smith AJ, Cramer EM, Zeng M, Masopust D, Carlis JV, Ran L, Vanderford TH, Paiardini M, Isett RB, Baldwin DA, Else JG, Staprans SI, Silvestri G, Haase AT, Kelvin DJ (2009) Global genomic analysis reveals rapid control of a robust innate response in SIV-infected sooty mangabeys. J Clin Invest 119:3556–3572

Bosinger SE, Sodora DL, Silvestri G (2011) Generalized immune activation and innate immune responses in simian immunodeficiency virus infection. Curr Opin HIV AIDS 6:411–418

Bouvet E, De Vincenzi I, Ancelle R, Vachon F (1989) Defloration as risk factor for heterosexual HIV transmission. Lancet 1:615

Bozzette SA, Berry SH, Duan N, Frankel MR, Leibowitz AA, Lefkowitz D, Emmons CA, Senterfitt JW, Berk ML, Morton SC, Shapiro MF (1998) The care of HIV-infected adults in the United States. HIV Cost and Services Utilization Study Consortium. N Engl J Med 339: 1897–1904

Brabin L (2002) Interactions of the female hormonal environment, susceptibility to viral infections, and disease progression. AIDS Patient Care STDS 16:211–221

Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L, Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC (2006) Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 12:1365–1371

Brettle RP, Raab GM, Ross A, Fielding KL, Gore SM, Bird AG (1995) HIV infection in women: immunological markers and the influence of pregnancy. AIDS 9:1177–1184

Brinkhof MW, Egger M, Boulle A, May M, Hosseinipour M, Sprinz E, Braitstein P, Dabis F, Reiss P, Bangsberg DR, Rickenbach M, Miro JM, Myer L, Mocroft A, Nash D, Keiser O, Pascoe M, Van Der Borght S, Schechter M (2007) Tuberculosis after initiation of antiretroviral therapy in low-income and high-income countries. Clin Infect Dis 45:1518–1521

Brown JM, Wald A, Hubbard A, Rungruengthanakit K, Chipato T, Rugpao S, Mmiro F, Celentano DD, Salata RS, Morrison CS, Richardson BA, Padian NS (2007) Incident and prevalent herpes simplex virus type 2 infection increases risk of HIV acquisition among women in Uganda and Zimbabwe. AIDS 21:1515–1523

Buchacz K, Rogol AD, Lindsey JC, Wilson CM, Hughes MD, Seage GR 3rd, Oleske JM, Rogers AS (2003) Delayed onset of pubertal development in children and adolescents with perinatally acquired HIV infection. J Acquir Immune Defic Syndr 33:56–65

Buggert M, Frederiksen J, Noyan K, Svard J, Barqasho B, Sonnerborg A, Lund O, Nowak P, Karlsson AC (2014) Multiparametric bioinformatics distinguish the Cd4/Cd8 ratio as a suitable laboratory predictor of combined T cell pathogenesis in HIV infection. J Immunol 192:2099–2108

Bulterys M, Chao A, Habimana P, Dushimimana A, Nawrocki P, Saah A (1994) Incident HIV-1 infection in a cohort of young women in Butare, Rwanda. AIDS 8:1585–1591

Bulterys M, Smith D, Chao A, Jaffe H (2007) Hormonal contraception and incident HIV-1 infection: new insight and continuing challenges. AIDS 21:97–99

Burger DM, Siebers MC, Hugen PW, Aarnoutse RE, Hekster YA, Koopmans PP (2002) Pharmacokinetic variability caused by gender: do women have higher indinavir exposure than men? J Acquir Immune Defic Syndr 29:101–102

Bush CE, Donovan RM, Markowitz N, Baxa D, Kvale P, Saravolatz LD (1996) Gender is not a factor in serum human immunodeficiency virus type 1 RNA levels in patients with viremia. J Clin Microbiol 34:970–972

Butler AR, Smith JA, Polis CB, Gregson S, Stanton D, Hallett TB (2013) Modelling the global competing risks of a potential interaction between injectable hormonal contraception and HIV risk. AIDS 27:105–113

Byrne J, Warburton D (1987) Male excess among anatomically normal fetuses in spontaneous abortions. Am J Med Genet 26:605–611

Cameron DW, Simonsen JN, D'costa LJ, Ronald AR, Maitha GM, Gakinya MN, Cheang M, Ndinya-Achola JO, Piot P, Brunham RC et al (1989) Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men. Lancet 2:403–407

Campillo-Gimenez L, Laforge M, Fay M, Brussel A, Cumont MC, Monceaux V, Diop O, Levy Y, Hurtrel B, Zaunders J, Corbeil J, Elbim C, Estaquier J (2010) Nonpathogenesis of simian immunodeficiency virus infection is associated with reduced inflammation and recruitment of plasmacytoid dendritic cells to lymph nodes, not to lack of an interferon type I response, during the acute phase. J Virol 84:1838–1846

Card CM, Mclaren PJ, Wachihi C, Kimani J, Plummer FA, Fowke KR (2009) Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of Cd4(+) Cd25(+)Foxp3(+) regulatory T cells. J Infect Dis 199:1318–1322

Carias AM, Mccoombe S, Mcraven M, Anderson M, Galloway N, Vandergrift N, Fought AJ, Lurain J, Duplantis M, Veazey RS, Hope TJ (2013) Defining the interaction of HIV-1 with the mucosal barriers of the female reproductive tract. J Virol 87:11388–11400

Carpenter LM, Kamali A, Ruberantwari A, Malamba SS, Whitworth JA (1999) Rates of HIV-1 transmission within marriage in rural Uganda in relation to the HIV sero-status of the partners. AIDS 13:1083–1089

Casper C, Carrell D, Miller KG, Judson FD, Meier AS, Pauk JS, Morrow RA, Corey L, Wald A, Celum C (2006) HIV serodiscordant sex partners and the prevalence of human herpesvirus 8 infection among HIV negative men who have sex with men: baseline data from the Explore Study. Sex Transm Infect 82:229–235

Casper C, Wald A, Pauk J, Tabet SR, Corey L, Celum CL (2002) Correlates of prevalent and incident Kaposi's sarcoma-associated herpesvirus infection in men who have sex with men. J Infect Dis 185:990–993

Castelo-Branco C, Cancelo MJ, Villero J, Nohales F, Julia MD (2005) Management of post-menopausal vaginal atrophy and atrophic vaginitis. Maturitas 52(Suppl 1):S46–S52

Cejtin HE, Jacobson L, Springer G, Watts DH, Levine A, Greenblatt R, Anastos K, Minkoff HL, Massad LS, Schmidt JB (2003) Effect of hormonal contraceptive use on plasma HIV-1-RNA levels among HIV-infected women. AIDS 17:1702–1704

Cejtin HE, Kalinowski A, Bacchetti P, Taylor RN, Watts DH, Kim S, Massad LS, Preston-Martin S, Anastos K, Moxley M, Minkoff HL (2006) Effects of human immunodeficiency virus on protracted amenorrhea and ovarian dysfunction. Obstet Gynecol 108:1423–1431

Cescon A, Patterson S, Chan K, Palmer AK, Margolese S, Burchell AN, Cooper C, Klein MB, Machouf N, Montaner JS, Tsoukas C, Hogg RS, Raboud JM, Loutfy MR (2013) Gender Differences in Clinical Outcomes among HIV-Positive Individuals on Antiretroviral Therapy in Canada: A Multisite Cohort Study. PLoS One 8:e83649

Chaisson RE, Keruly JC, Moore RD (1995) Race, sex, drug use, and progression of human immunodeficiency virus disease. N Engl J Med 333:751–756

Chakrabarti LA, Simon V (2010) Immune mechanisms of HIV control. Curr Opin Immunol 22: 488–496

Chakraborty H, Sen PK, Helms RW, Vernazza PL, Fiscus SA, Eron JJ, Patterson BK, Coombs RW, Krieger JN, Cohen MS (2001) Viral burden in genital secretions determines male-to-female sexual transmission of HIV-1: a probabilistic empiric model. AIDS 15: 621–627

Chang JJ, Woods M, Lindsay RJ, Doyle EH, Griesbeck M, Chan ES, Robbins GK, Bosch RJ, Altfeld M (2013) Higher expression of several interferon-stimulated genes in HIV-1-infected females after adjusting for the level of viral replication. J Infect Dis 208:830–838

Chang LW, Osei-Kwasi M, Boakye D, Aidoo S, Hagy A, Curran JW, Vermund SH (2002) HIV-1 and HIV-2 seroprevalence and risk factors among hospital outpatients in the Eastern Region of Ghana, West Africa. J Acquir Immune Defic Syndr 29:511–516

Chantry CJ, Frederick MM, Meyer WA 3rd, Handelsman E, Rich K, Paul ME, Diaz C, Cooper ER, Foca M, Adeniyi-Jones SK, Moye J (2007) Endocrine abnormalities and impaired growth in human immunodeficiency virus-infected children. Pediatr Infect Dis J 26:53–60

Chege D, Chai Y, Huibner S, Kain T, Wachihi C, Kimani M, Barasa S, Mckinnon LR, Muriuki FK, Kariri A, Jaoko W, Anzala O, Kimani J, Ball TB, Plummer FA, Kaul R (2012) Blunted Il17/

Il22 and pro-inflammatory cytokine responses in the genital tract and blood of HIV-exposed, seronegative female sex workers in Kenya. PLoS One 7:e43670
Cherpes TL, Busch JL, Sheridan BS, Harvey SA, Hendricks RL (2008) Medroxyprogesterone acetate inhibits Cd8+ T cell viral-specific effector function and induces herpes simplex virus type 1 reactivation. J Immunol 181:969–975
Chirgwin KD, Feldman J, Muneyyirci-Delale O, Landesman S, Minkoff H (1996) Menstrual function in human immunodeficiency virus-infected women without acquired immunodeficiency syndrome. J Acquir Immune Defic Syndr Hum Retrovirol 12:489–494
Chohan B, Lavreys L, Rainwater SM, Overbaugh J (2005) Evidence for frequent reinfection with human immunodeficiency virus type 1 of a different subtype. J Virol 79:10701–10708
Choi HH, Gray PB, Storer TW, Calof OM, Woodhouse L, Singh AB, Padero C, Mac RP, Sinha-Hikim I, Shen R, Dzekov J, Dzekov C, Kushnir MM, Rockwood AL, Meikle AW, Lee ML, Hays RD, Bhasin S (2005) Effects of testosterone replacement in human immunodeficiency virus-infected women with weight loss. J Clin Endocrinol Metab 90:1531–1541
Chokunonga E, Borok MZ, Chirenje ZM, Nyakabau AM, Parkin DM (2013) Trends in the incidence of cancer in the black population of Harare, Zimbabwe 1991-2010. Int J Cancer 133:721–729
Chomont N, Hocini H, Gresenguet G, Brochier C, Bouhlal H, Andreoletti L, Becquart P, Charpentier C, De Dieu Longo J, Si-Mohamed A, Kazatchkine MD, Belec L (2007) Early arcHIVes of genetically-restricted proviral Dna in the female genital tract after heterosexual transmission of HIV-1. AIDS 21:153–162
Chu SY, Buehler JW, Berkelman RL (1990) Impact of the human immunodeficiency virus epidemic on mortality in women of reproductive age, United States. JAMA 264:225–229
Clark R (2005) Sex differences in antiretroviral therapy-associated intolerance and adverse events. Drug Saf 28:1075–1083
Clavel F, Guetard D, Brun-Vezinet F, Chamaret S, Rey MA, Santos-Ferreira MO, Laurent AG, Dauguet C, Katlama C, Rouzioux C et al (1986) Isolation of a new human retrovirus from West African patients with Aids. Science 233:343–346
Clayton JA, Collins FS (2014) Policy: NIH to balance sex in cell and animal studies. Nature 509: 282–283
Clemetson DB, Moss GB, Willerford DM, Hensel M, Emonyi W, Holmes KK, Plummer F, Ndinya-Achola J, Roberts PL, Hillier S et al (1993) Detection of HIV DNA in cervical and vaginal secretions. Prevalence and correlates among women in Nairobi, Kenya. JAMA 269: 2860–2864
Clifford GM, Franceschi S (2009) Cancer risk in HIV-infected persons: influence of Cd4(+) count. Future Oncol 5:669–678
Cohen MS, Chen YQ, Mccauley M, Gamble T, Hosseinipour MC, Kumarasamy N, Hakim JG, Kumwenda J, Grinsztejn B, Pilotto JH, Godbole SV, Mehendale S, Chariyalertsak S, Santos BR, Mayer KH, Hoffman IF, Eshleman SH, Piwowar-Manning E, Wang L, Makhema J, Mills LA, De Bruyn G, Sanne I, Eron J, Gallant J, Havlir D, Swindells S, Ribaudo H, Elharrar V, Burns D, Taha TE, Nielsen-Saines K, Celentano D, Essex M, Fleming TR (2011) Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med 365:493–505
Cohen MS, Hoffman IF, Royce RA, Kazembe P, Dyer JR, Daly CC, Zimba D, Vernazza PL, Maida M, Fiscus SA, Eron JJ Jr (1997) Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. Lancet 349:1868–1873
Cohen MS, Mccauley M, Gamble TR (2012) HIV treatment as prevention and HPTN 052. Curr Opin HIV AIDS 7:99–105
Collazos J, Asensi V, Carton JA (2007) Sex differences in the clinical, immunological and virological parameters of HIV-infected patients treated with HAART. AIDS 21:835–843

Collazos J, Carton JA, Asensi V (2011) Gender differences in liver fibrosis and hepatitis C virus-related parameters in patients coinfected with human immunodeficiency virus. Curr HIV Res 9:339–345

Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, O'sullivan MJ, Vandyke R, Bey M, Shearer W, Jacobson RL et al (1994) Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric Aids Clinical Trials Group Protocol 076 Study Group. N Engl J Med 331:1173–1180

Cooley TP, Hirschhorn LR, O'keane JC (1996) Kaposi's sarcoma in women with AIDS. AIDS 10: 1221–1225

Cornell M, Schomaker M, Garone DB, Giddy J, Hoffmann CJ, Lessells R, Maskew M, Prozesky H, Wood R, Johnson LF, Egger M, Boulle A, Myer L (2012) Gender differences in survival among adult patients starting antiretroviral therapy in South Africa: a multicentre cohort study. PLoS Med 9:e1001304

Cornell M, Technau K, Fairall L, Wood R, Moultrie H, Van Cutsem G, Giddy J, Mohapi L, Eley B, Macphail P, Prozesky H, Rabie H, Davies MA, Maxwell N, Boulle A (2009) Monitoring the South African National Antiretroviral Treatment Programme, 2003-2007: the IEDEA Southern Africa collaboration. S Afr Med J 99:653–660

Coutsoudis A, Dabis F, Fawzi W, Gaillard P, Haverkamp G, Harris DR, Jackson JB, Leroy V, Meda N, Msellati P, Newell ML, Nsuati R, Read JS, Wiktor S (2004) Late postnatal transmission of HIV-1 in breast-fed children: an individual patient data meta-analysis. J Infect Dis 189: 2154–2166

Coutsoudis A, Pillay K, Kuhn L, Spooner E, Tsai WY, Coovadia HM (2001) Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: prospective cohort study from Durban, South Africa. AIDS 15:379–387

Cozzi Lepri A, Pezzotti P, Dorrucci M, Phillips AN, Rezza G (1994) HIV disease progression in 854 women and men infected through injecting drug use and heterosexual sex and followed for up to nine years from seroconversion. Italian Seroconversion Study. BMJ 309:1537–1542

Critchlow CW, Wolner-Hanssen P, Eschenbach DA, Kiviat NB, Koutsky LA, Stevens CE, Holmes KK (1995) Determinants of cervical ectopia and of cervicitis: age, oral contraception, specific cervical infection, smoking, and douching. Am J Obstet Gynecol 173:534–543

Csajka C, Marzolini C, Fattinger K, Decosterd LA, Telenti A, Biollaz J, Buclin T (2004) Population pharmacokinetics of indinavir in patients infected with human immunodeficiency virus. Antimicrob Agents Chemother 48:3226–3232

Cu-Uvin S, Hogan JW, Caliendo AM, Harwell J, Mayer KH, Carpenter CC (2001) Association between bacterial vaginosis and expression of human immunodeficiency virus type 1 RNA in the female genital tract. Clin Infect Dis 33:894–896

Cu-Uvin S, Ko H, Jamieson DJ, Hogan JW, Schuman P, Anderson J, Klein RS (2002) Prevalence, incidence, and persistence or recurrence of trichomoniasis among human immunodeficiency virus (HIV)-positive women and among HIV-negative women at high risk for HIV infection. Clin Infect Dis 34:1406–1411

Currier J, Averitt Bridge D, Hagins D, Zorrilla CD, Feinberg J, Ryan R, Falcon R, Tennenberg A, Mrus J, Squires K (2010) Sex-based outcomes of darunavir-ritonavir therapy: a single-group trial. Ann Intern Med 153:349–357

Curtis KM, Nanda K, Kapp N (2009) Safety of hormonal and intrauterine methods of contraception for women with HIV/Aids: a systematic review. AIDS 23(Suppl 1):S55–S67

Da Silva ZJ, Oliveira I, Andersen A, Dias F, Rodrigues A, Holmgren B, Andersson S, Aaby P (2008) Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? AIDS 22:1195–1202

Datta SD, Torrone E, Kruszon-Moran D, Berman S, Johnson R, Satterwhite CL, Papp J, Weinstock H (2012) Chlamydia trachomatis trends in the United States among persons 14 to 39 years of age, 1999-2008. Sex Transm Dis 39:92–96

Dauby N, De Wit S, Delforge M, Necsoi VC, Clumeck N (2011) Characteristics of non-Aids-defining malignancies in the Haart era: a clinico-epidemiological study. J Int AIDS Soc 14:16

De Cock KM, Adjorlolo G, Ekpini E, Sibailly T, Kouadio J, Maran M, Brattegaard K, Vetter KM, Doorly R, Gayle HD (1993) Epidemiology and transmission of HIV-2. Why there is no HIV-2 pandemic. JAMA 270:2083–2086

De Cock KM, Fowler MG, Mercier E, De Vincenzi I, Saba J, Hoff E, Alnwick DJ, Rogers M, Shaffer N (2000) Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. JAMA 283:1175–1182

De Martino M, Tovo PA, Galli L, Gabiano C, Chiarelli F, Zappa M, Gattinara GC, Bassetti D, Giacomet V, Chiappini E, Duse M, Garetto S, Caselli D (2001) Puberty in perinatal HIV-1 infection: a multicentre longitudinal study of 212 children. AIDS 15:1527–1534

De Pommerol M, Hessamfar M, Lawson-Ayayi S, Neau D, Geffard S, Farbos S, Uwamaliya B, Vandenhende MA, Pellegrin JL, Blancpain S, Dabis F, Morlat P (2011) Menopause and HIV infection: age at onset and associated factors, Anrs Co3 Aquitaine cohort. Int J STD AIDS 22: 67–72

De Vincenzi I (1994) A longitudinal study of human immunodeficiency virus transmission by heterosexual partners. European Study Group on Heterosexual Transmission of HIV. N Engl J Med 331:341–346

De Walque D, Nakiyingi-Miiro JS, Busingye J, Whitworth JA (2005) Changing association between schooling levels and HIV-1 infection over 11 years in a rural population cohort in south-west Uganda. Trop Med Int Health 10:993–1001

Delmas MC, Jadand C, De Vincenzi I, Deveau C, Persoz A, Sobel A, Kazatchkine M, Brunet JB, Meyer L (1997) Gender difference in Cd4+ cell counts persist after HIV-1 infection. Seroco Study Group. AIDS 11:1071–1073

Deluca A, Chaisson RE, Martinson NA (2009) Intensified case finding for tuberculosis in prevention of mother-to-child transmission programs: a simple and potentially vital addition for maternal and child health. J Acquir Immune Defic Syndr 50:196–199

Derdeyn CA, Decker JM, Bibollet-Ruche F, Mokili JL, Muldoon M, Denham SA, Heil ML, Kasolo F, Musonda R, Hahn BH, Shaw GM, Korber BT, Allen S, Hunter E (2004) Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. Science 303: 2019–2022

Derdeyn CA, Hunter E (2008) Viral characteristics of transmitted HIV. Curr Opin HIV AIDS 3: 16–21

Dieffenbach CW (2012) Preventing HIV transmission through antiretroviral treatment-mediated virologic suppression: aspects of an emerging scientific agenda. Curr Opin HIV AIDS 7: 106–110

Ding J, Rapista A, Teleshova N, Mosoyan G, Jarvis GA, Klotman ME, Chang TL (2010) Neisseria gonorrhoeae enhances HIV-1 infection of primary resting Cd4+ T cells through Tlr2 activation. J Immunol 184:2814–2824

Diop OM, Pison G, Diouf I, Enel C, Lagarde E (2000) Incidence of HIV-1 and HIV-2 infections in a rural community in southern Senegal. AIDS 14:1671–1672

Dolan Looby SE, Collins M, Lee H, Grinspoon S (2009) Effects of long-term testosterone administration in HIV-infected women: a randomized, placebo-controlled trial. AIDS 23: 951–959

Dolan S, Wilkie S, Aliabadi N, Sullivan MP, Basgoz N, Davis B, Grinspoon S (2004) Effects of testosterone administration in human immunodeficiency virus-infected women with low weight: a randomized placebo-controlled study. Arch Intern Med 164:897–904

Dominguez F, Galan A, Martin JJ, Remohi J, Pellicer A, Simon C (2003) Hormonal and embryonic regulation of chemokine receptors Cxcr1, Cxcr4, Ccr5 and Ccr2B in the human endometrium and the human blastocyst. Mol Hum Reprod 9:189–198

Donnelly CA, Bartley LM, Ghani AC, Le Fevre AM, Kwong GP, Cowling BJ, Van Sighem AI, De Wolf F, Rode RA, Anderson RM (2005) Gender difference in HIV-1 RNA viral loads. HIV Med 6:170–178

Douglas JL, Gustin JK, Viswanathan K, Mansouri M, Moses AV, Fruh K (2010) The great escape: viral strategies to counter Bst-2/tetherin. PLoS Pathog 6:e1000913

Egger M, May M, Chene G, Phillips AN, Ledergerber B, Dabis F, Costagliola D, D'arminio Monforte A, De Wolf F, Reiss P, Lundgren JD, Justice AC, Staszewski S, Leport C, Hogg RS, Sabin CA, Gill MJ, Salzberger B, Sterne JA (2002) Prognosis of HIV-1-infected patients

starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. Lancet 360:119–129

El-Sadr WM, Coburn BJ, Blower S (2011) Modeling the impact on the HIV epidemic of treating discordant couples with antiretrovirals to prevent transmission. AIDS 25:2295–2299

El-Sadr WM, Lundgren J, Neaton JD, Gordin F, Abrams D, Arduino RC, Babiker A, Burman W, Clumeck N, Cohen CJ, Cohn D, Cooper D, Darbyshire J, Emery S, Fatkenheuer G, Gazzard B, Grund B, Hoy J, Klingman K, Losso M, Markowitz N, Neuhaus J, Phillips A, Rappoport C (2006) Cd4+ count-guided interruption of antiretroviral treatment. N Engl J Med 355: 2283–2296

Eltom MA, Jemal A, Mbulaiteye SM, Devesa SS, Biggar RJ (2002) Trends in Kaposi's sarcoma and non-Hodgkin's lymphoma incidence in the United States from 1973 through 1998. J Natl Cancer Inst 94:1204–1210

Emery J, Pick N, Mills EJ, Cooper CL (2010) Gender differences in clinical, immunological, and virological outcomes in highly active antiretroviral-treated HIV-HCV coinfected patients. Patient Prefer Adherence 4:97–103

Enomoto LM, Kloberdanz KJ, Mack DG, Elizabeth D, Weinberg A (2007) Ex vivo effect of estrogen and progesterone compared with dexamethasone on cell-mediated immunity of HIV-infected and uninfected subjects. J Acquir Immune Defic Syndr 45:137–143

Estes JD, Gordon SN, Zeng M, Chahroudi AM, Dunham RM, Staprans SI, Reilly CS, Silvestri G, Haase AT (2008) Early resolution of acute immune activation and induction of Pd-1 in SIV-infected sooty mangabeys distinguishes nonpathogenic from pathogenic infection in rhesus macaques. J Immunol 180:6798–6807

Evans JS, Nims T, Cooley J, Bradley W, Jagodzinski L, Zhou S, Melcher GP, Burke DS, Vahey M (1997) Serum levels of virus burden in early-stage human immunodeficiency virus type 1 disease in women. J Infect Dis 175:795–800

Exner TM, Dworkin SL, Hoffman S, Ehrhardt AA (2003) Beyond the male condom: the evolution of gender-specific HIV interventions for women. Annu Rev Sex Res 14:114–136

Fabricatore AN, Wadden TA, Moore RH, Butryn ML, Gravallese EA, Erondu NE, Heymsfield SB, Nguyen AM (2009) Attrition from randomized controlled trials of pharmacological weight loss agents: a systematic review and analysis. Obes Rev 10:333–341

Farage M, Maibach H (2006) Lifetime changes in the vulva and vagina. Arch Gynecol Obstet 273: 195–202

Fardet L, Mary-Krause M, Heard I, Partisani M, Costagliola D (2006) Influence of gender and HIV transmission group on initial highly active antiretroviral therapy prescription and treatment response. HIV Med 7:520–529

Farzadegan H, Hoover DR, Astemborski J, Lyles CM, Margolick JB, Markham RB, Quinn TC, Vlahov D (1998) Sex differences in HIV-1 viral load and progression to Aids. Lancet 352: 1510–1514

Favre D, Mold J, Hunt PW, Kanwar B, Loke P, Seu L, Barbour JD, Lowe MM, Jayawardene A, Aweeka F, Huang Y, Douek DC, Brenchley JM, Martin JN, Hecht FM, Deeks SG, Mccune JM (2010) Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of Th17 to regulatory T cells in HIV disease. Sci Transl Med 2:32ra36

Fenner L, Forster M, Boulle A, Phiri S, Braitstein P, Lewden C, Schechter M, Kumarasamy N, Pascoe M, Sprinz E, Bangsberg DR, Sow PS, Dickinson D, Fox MP, Mcintyre J, Khongphatthanayothin M, Dabis F, Brinkhof MW, Wood R, Egger M (2011) Tuberculosis in HIV programmes in lower-income countries: practices and risk factors. Int J Tuberc Lung Dis 15:620–627

Fernandez MI, Pedron T, Tournebize R, Olivo-Marin JC, Sansonetti PJ, Phalipon A (2003) Anti-inflammatory role for intracellular dimeric immunoglobulin a by neutralization of lipopolysaccharide in epithelial cells. Immunity 18:739–749

Ferradini L, Jeannin A, Pinoges L, Izopet J, Odhiambo D, Mankhambo L, Karungi G, Szumilin E, Balandine S, Fedida G, Carrieri MP, Spire B, Ford N, Tassie JM, Guerin PJ, Brasher C (2006) Scaling up of highly active antiretroviral therapy in a rural district of Malawi: an effectiveness assessment. Lancet 367:1335–1342

Ferreira CE, Pinto-Neto AM, Conde DM, Costa-Paiva L, Morais SS, Magalhaes J (2007) Menopause symptoms in women infected with HIV: prevalence and associated factors. Gynecol Endocrinol 23:198–205

Fideli US, Allen SA, Musonda R, Trask S, Hahn BH, Weiss H, Mulenga J, Kasolo F, Vermund SH, Aldrovandi GM (2001) Virologic and immunologic determinants of heterosexual transmission of human immunodeficiency virus type 1 in Africa. AIDS Res Hum Retroviruses 17:901–910

Figueroa JP, Brathwaite A, Morris J, Ward E, Peruga A, Blattner W, Vermund SH, Hayes R (1994) Rising HIV-1 prevalence among sexually transmitted disease clinic attenders in Jamaica: traumatic sex and genital ulcers as risk factors. J Acquir Immune Defic Syndr 7:310–316

Finkel DG, John G, Holland B, Slim J, Smith SM (2003) Women have a greater immunological response to effective virological HIV-1 therapy. AIDS 17:2009–2011

Fischl MA, Dickinson GM, Scott GB, Klimas N, Fletcher MA, Parks W (1987) Evaluation of heterosexual partners, children, and household contacts of adults with Aids. JAMA 257: 640–644

Fiscus SA, Cu-Uvin S, Eshete AT, Hughes MD, Bao Y, Hosseinipour M, Grinsztejn B, Badal-Faesen S, Dragavon J, Coombs RW, Braun K, Moran L, Hakim J, Flanigan T, Kumarasamy N, Campbell TB (2013) Changes in HIV-1 subtypes B and C genital tract Rna in women and men after initiation of antiretroviral therapy. Clin Infect Dis 57:290–297

Fish EN (2008) The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol 8:737–744

Fleishman JA, Yehia BR, Moore RD, Gebo KA, Agwu AL (2012) Disparities in receipt of antiretroviral therapy among HIV-infected adults (2002-2008). Med Care 50:419–427

Fleming DT, Wasserheit JN (1999) From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex Transm Infect 75:3–17

Floridia M, Giuliano M, Palmisano L, Vella S (2008) Gender differences in the treatment of HIV infection. Pharmacol Res 58:173–182

Forbi JC, Agwale SM (2009) Inverted CD4+/Cd8+ ratio associated with Aids event and death in HIV-1 infected individuals in Nasarawa State, Nigeria. Tanzan J Health Res 11:144–148

Franklin RD, Kutteh WH (1999) Characterization of immunoglobulins and cytokines in human cervical mucus: influence of exogenous and endogenous hormones. J Reprod Immunol 42:93–106

Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ (2006) Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. AIDS 20:73–83

Friedland GH, Saltzman B, Vileno J, Freeman K, Schrager LK, Klein RS (1991) Survival differences in patients with AIDS. J Acquir Immune Defic Syndr 4:144–153

Frost SD, Wrin T, Smith DM, Kosakovsky Pond SL, Liu Y, Paxinos E, Chappey C, Galovich J, Beauchaine J, Petropoulos CJ, Little SJ, Richman DD (2005) Neutralizing antibody responses drive the evolution of human immunodeficiency virus type 1 envelope during recent HIV infection. Proc Natl Acad Sci U S A 102:18514–18519

Furth PA, Westphal H, Hennighausen L (1990) Expression from the HIV-LTR is stimulated by glucocorticoids and pregnancy. AIDS Res Hum Retroviruses 6:553–560

Fylkesnes K, Musonda RM, Kasumba K, Ndhlovu Z, Mluanda F, Kaetano L, Chipaila CC (1997) The HIV epidemic in Zambia: socio-demographic prevalence patterns and indications of trends among childbearing women. AIDS 11:339–345

Galli L, Puliti D, Chiappini E, Gabiano C, Tovo PA, Pezzotti P, De Martino M (2005) Lower mother-to-child HIV-1 transmission in boys is independent of type of delivery and antiretroviral prophylaxis: the Italian Register for HIV Infection in Children. J Acquir Immune Defic Syndr 40:479–485

Gandhi M, Bacchetti P, Miotti P, Quinn TC, Veronese F, Greenblatt RM (2002) Does patient sex affect human immunodeficiency virus levels? Clin Infect Dis 35:313–322

Gandhi RT, Spritzler J, Chan E, Asmuth DM, Rodriguez B, Merigan TC, Hirsch MS, Shafer RW, Robbins GK, Pollard RB (2006) Effect of baseline- and treatment-related factors on

immunologic recovery after initiation of antiretroviral therapy in HIV-1-positive subjects: results from Actg 384. J Acquir Immune Defic Syndr 42:426–434
Garcia-Moreno C, Watts C (2000) Violence against women: its importance for HIV/Aids. AIDS 14(Suppl 3):S253–S265
Garenne M (2002) Sex ratios at birth in African populations: a review of survey data. Hum Biol 74:889–900
Genesca M, Li J, Fritts L, Chohan P, Bost K, Rourke T, Blozis SA, Mcchesney MB, Miller CJ (2007) Depo-Provera abrogates attenuated lentivirus-induced protection in male rhesus macaques challenged intravenously with pathogenic Sivmac239. J Med Primatol 36:266–275
Genesca M, Mcchesney MB, Miller CJ (2010) Depo-provera treatment does not abrogate protection from intravenous SIV challenge in female macaques immunized with an attenuated Aids virus. PLoS One 5:e9814
Gertner JM, Kaufman FR, Donfield SM, Sleeper LA, Shapiro AD, Howard C, Gomperts ED, Hilgartner MW (1994) Delayed somatic growth and pubertal development in human immunodeficiency virus-infected hemophiliac boys: Hemophilia Growth and Development Study. J Pediatr 124:896–902
Getahun H, Gunneberg C, Granich R, Nunn P (2010) HIV infection-associated tuberculosis: the epidemiology and the response. Clin Infect Dis 50(Suppl 3):S201–S207
Ghanem KG, Shah N, Klein RS, Mayer KH, Sobel JD, Warren DL, Jamieson DJ, Duerr AC, Rompalo AM (2005) Influence of sex hormones, HIV status, and concomitant sexually transmitted infection on cervicovaginal inflammation. J Infect Dis 191:358–366
Ghys PD, Zaba B, Prins M (2007) Survival and mortality of people infected with HIV in low and middle income countries: results from the extended Alpha network. AIDS 21(Suppl 6):S1–S4
Gilad J, Walfisch A, Borer A, Schlaeffer F (2003) Gender differences and sex-specific manifestations associated with human immunodeficiency virus infection in women. Eur J Obstet Gynecol Reprod Biol 109:199–205
Gilbert P, Wang M, Wrin T, Petropoulos C, Gurwith M, Sinangil F, D'souza P, Rodriguez-Chavez IR, Decamp A, Giganti M, Berman PW, Self SG, Montefiori DC (2010) Magnitude and breadth of a nonprotective neutralizing antibody response in an efficacy trial of a candidate HIV-1 gp120 vaccine. J Infect Dis 202:595–605
Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, Gurwith M, Heyward WL, Jobes DV, Popovic V, Self SG, Sinangil F, Burke D, Berman PW (2005) Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. J Infect Dis 191:666–677
Gillgrass AE, Ashkar AA, Rosenthal KL, Kaushic C (2003) Prolonged exposure to progesterone prevents induction of protective mucosal responses following intravaginal immunization with attenuated herpes simplex virus type 2. J Virol 77:9845–9851
Giorgi JV, Hultin LE, Mckeating JA, Johnson TD, Owens B, Jacobson LP, Shih R, Lewis J, Wiley DJ, Phair JP, Wolinsky SM, Detels R (1999) Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J Infect Dis 179:859–870
Gisselquist D (2008) Comment on "hormonal contraception and HIV prevalence in four African countries". Contraception 78:346, Author reply 346–347
Glynn JR, Carael M, Auvert B, Kahindo M, Chege J, Musonda R, Kaona F, Buve A (2001) Why do young women have a much higher prevalence of HIV than young men? A study in Kisumu, Kenya and Ndola, Zambia. AIDS 15(Suppl 4):S51–S60
Goedert JJ, Schairer C, Mcneel TS, Hessol NA, Rabkin CS, Engels EA (2006) Risk of breast, ovary, and uterine corpus cancers among 85,268 women with Aids. Br J Cancer 95:642–648
Gohier B, Goeb JL, Rannou-Dubas K, Fouchard I, Cales P, Garre JB (2003) Hepatitis C, alpha interferon, anxiety and depression disorders: a prospective study of 71 patients. World J Biol Psychiatry 4:115–118
Gonzalez VD, Falconer K, Blom KG, Reichard O, Morn B, Laursen AL, Weis N, Alaeus A, Sandberg JK (2009) High levels of chronic immune activation in the T-cell compartments of patients coinfected with hepatitis C virus and human immunodeficiency virus type 1 and on

highly active antiretroviral therapy are reverted by alpha interferon and ribavirin treatment. J Virol 83:11407–11411

Gouveia NL, Camargo M, Caseiro MM, Janini LM, Sucupira MC, Diaz RS (2014) Homogenous HIV-1 subtype B quasispecies in Brazilian men and women recently infected via heterosexual transmission. Virus Genes 48:421–428

Graham CS, Baden LR, Yu E, Mrus JM, Carnie J, Heeren T, Koziel MJ (2001) Influence of human immunodeficiency virus infection on the course of hepatitis C virus infection: a meta-analysis. Clin Infect Dis 33:562–569

Gratwohl A, Hermans J, Niederwieser D, Van Biezen A, Van Houwelingen HC, Apperley J (2001) Female donors influence transplant-related mortality and relapse incidence in male recipients of sibling blood and marrow transplants. Hematol J 2:363–370

Gray G, Berger P (2007) Pain in women with HIV/Aids. Pain 132(Suppl 1):S13–S21

Gray RH (2012) Use of hormonal contraceptives and risk of HIV-1 transmission. Lancet Infect Dis 12:507, Author reply 510–511

Gray RH, Kiwanuka N, Quinn TC, Sewankambo NK, Serwadda D, Mangen FW, Lutalo T, Nalugoda F, Kelly R, Meehan M, Chen MZ, Li C, Wawer MJ (2000) Male circumcision and HIV acquisition and transmission: cohort studies in Rakai, Uganda. Rakai Project Team. AIDS 14:2371–2381

Gray RH, Li X, Kigozi G, Serwadda D, Brahmbhatt H, Wabwire-Mangen F, Nalugoda F, Kiddugavu M, Sewankambo N, Quinn TC, Reynolds SJ, Wawer MJ (2005) Increased risk of incident HIV during pregnancy in Rakai, Uganda: a prospective study. Lancet 366:1182–1188

Gray RH, Wawer MJ, Brookmeyer R, Sewankambo NK, Serwadda D, Wabwire-Mangen F, Lutalo T, Li X, Vancott T, Quinn TC (2001) Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. Lancet 357: 1149–1153

Gray RH, Wawer MJ, Sewankambo NK, Serwadda D, Li C, Moulton LH, Lutalo T, Wabwire-Mangen F, Meehan MP, Ahmed S, Paxton LA, Kiwanuka N, Nalugoda F, Korenromp EL, Quinn TC (1999) Relative risks and population attributable fraction of incident HIV associated with symptoms of sexually transmitted diseases and treatable symptomatic sexually transmitted diseases in Rakai District, Uganda. Rakai Project Team. AIDS 13:2113–2123

Gray-Swain MR, Peipert JF (2006) Pelvic inflammatory disease in adolescents. Curr Opin Obstet Gynecol 18:503–510

Grebely J, Page K, Sacks-Davis R, Van Der Loeff MS, Rice TM, Bruneau J, Morris MD, Hajarizadeh B, Amin J, Cox AL, Kim AY, Mcgovern BH, Schinkel J, George J, Shoukry NH, Lauer GM, Maher L, Lloyd AR, Hellard M, Dore GJ, Prins M (2014) The effects of female sex, viral genotype, and Il28B genotype on spontaneous clearance of acute hepatitis C virus infection. Hepatology 59:109–120

Grebely J, Raffa JD, Lai C, Krajden M, Conway B, Tyndall MW (2007) Factors associated with spontaneous clearance of hepatitis C virus among illicit drug users. Can J Gastroenterol 21: 447–451

Greenblatt RM, Ameli N, Grant RM, Bacchetti P, Taylor RN (2000) Impact of the ovulatory cycle on virologic and immunologic markers in HIV-infected women. J Infect Dis 181:82–90

Greenspan D, Komaroff E, Redford M, Phelan JA, Navazesh M, Alves ME, Kamrath H, Mulligan R, Barr CE, Greenspan JS (2000) Oral mucosal lesions and HIV viral load in the Women's Interagency HIV Study (Wihs). J Acquir Immune Defic Syndr 25:44–50

Grinspoon S, Corcoran C, Askari H, Schoenfeld D, Wolf L, Burrows B, Walsh M, Hayden D, Parlman K, Anderson E, Basgoz N, Klibanski A (1998a) Effects of androgen administration in men with the Aids wasting syndrome. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 129:18–26

Grinspoon S, Corcoran C, Miller K, Biller BM, Askari H, Wang E, Hubbard J, Anderson EJ, Basgoz N, Heller HM, Klibanski A (1997) Body composition and endocrine function in women with acquired immunodeficiency syndrome wasting. J Clin Endocrinol Metab 82: 1332–1337

Grinspoon S, Corcoran C, Stanley T, Katznelson L, Klibanski A (1998b) Effects of androgen administration on the growth hormone-insulin-like growth factor I axis in men with acquired immunodeficiency syndrome wasting. J Clin Endocrinol Metab 83:4251–4256

Grinspoon S, Corcoran C, Stanley T, Rabe J, Wilkie S (2001) Mechanisms of androgen deficiency in human immunodeficiency virus-infected women with the wasting syndrome. J Clin Endocrinol Metab 86:4120–4126

Grinsztejn B, Smeaton L, Barnett R, Klingman K, Hakim J, Flanigan T, Kumarasamy N, Campbell T, Currier J (2011) Sex-associated differences in pre-antiretroviral therapy plasma HIV-1 Rna in diverse areas of the world vary by Cd4(+) T-cell count. Antivir Ther 16: 1057–1062

Grosskurth H, Mosha F, Todd J, Mwijarubi E, Klokke A, Senkoro K, Mayaud P, Changalucha J, Nicoll A, Ka-Gina G et al (1995) Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. Lancet 346:530–536

Guay LA, Musoke P, Fleming T, Bagenda D, Allen M, Nakabiito C, Sherman J, Bakaki P, Ducar C, Deseyve M, Emel L, Mirochnick M, Fowler MG, Mofenson L, Miotti P, Dransfield K, Bray D, Mmiro F, Jackson JB (1999) Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVnet 012 randomised trial. Lancet 354:795–802

Guimaraes MD, Munoz A, Boschi-Pinto C, Castilho EA (1995) HIV infection among female partners of seropositive men in Brazil. Rio de Janeiro Heterosexual Study Group. Am J Epidemiol 142:538–547

Guimaraes MD, Vlahov D, Castilho EA (1997) Postcoital vaginal bleeding as a risk factor for transmission of the human immunodeficiency virus in a heterosexual partner study in Brazil. Rio de Janeiro Heterosexual Study Group. Arch Intern Med 157:1362–1368

Gupta P, Mellors J, Kingsley L, Riddler S, Singh MK, Schreiber S, Cronin M, Rinaldo CR (1997) High viral load in semen of human immunodeficiency virus type 1-infected men at all stages of disease and its reduction by therapy with protease and nonnucleoside reverse transcriptase inhibitors. J Virol 71:6271–6275

Haaland RE, Hawkins PA, Salazar-Gonzalez J, Johnson A, Tichacek A, Karita E, Manigart O, Mulenga J, Keele BF, Shaw GM, Hahn BH, Allen SA, Derdeyn CA, Hunter E (2009) Inflammatory genital infections mitigate a severe genetic bottleneck in heterosexual transmission of subtype A and C HIV-1. PLoS Pathog 5:e1000274

Haase AT (1999) Population biology of HIV-1 infection: viral and Cd4+ T cell demographics and dynamics in lymphatic tissues. Annu Rev Immunol 17:625–656

Haase AT (2010) Targeting early infection to prevent HIV-1 mucosal transmission. Nature 464: 217–223

Haase AT, Henry K, Zupancic M, Sedgewick G, Faust RA, Melroe H, Cavert W, Gebhard K, Staskus K, Zhang ZQ, Dailey PJ, Balfour HH Jr, Erice A, Perelson AS (1996) Quantitative image analysis of HIV-1 infection in lymphoid tissue. Science 274:985–989

Habib AG (2009) A clinical and epidemiologic update on the interaction between tuberculosis and human immunodeficiency virus infection in adults. Ann Afr Med 8:147–155

Hajizadeh M, Sia D, Heymann SJ, Nandi A (2014) Socioeconomic inequalities in HIV/Aids prevalence in sub-Saharan African countries: evidence from the Demographic Health Surveys. Int J Equity Health 13:18

Hall HI, Mcdavid K, Ling Q, Sloggett A (2006) Determinants of progression to Aids or death after HIV diagnosis, United States, 1996 to 2001. Ann Epidemiol 16:824–833

Hamel DJ, Sankale JL, Eisen G, Meloni ST, Mullins C, Gueye-Ndiaye A, Mboup S, Kanki PJ (2007) Twenty years of prospective molecular epidemiology in Senegal: changes in HIV diversity. AIDS Res Hum Retroviruses 23:1189–1196

Hanna L (1999) The menstrual cycle and viral load. BETA 12:18

Hargreaves JR, Bonell CP, Morison LA, Kim JC, Phetla G, Porter JD, Watts C, Pronyk PM (2007) Explaining continued high HIV prevalence in South Africa: socioeconomic factors, HIV incidence and sexual behaviour change among a rural cohort, 2001-2004. AIDS 21(Suppl 7):S39–S48

Hargreaves JR, Glynn JR (2002) Educational attainment and HIV-1 infection in developing countries: a systematic review. Trop Med Int Health 7:489–498

Harkonen PL, Vaananen HK (2006) Monocyte-macrophage system as a target for estrogen and selective estrogen receptor modulators. Ann NY Acad Sci 1089:218–227

Harris C, Small CB, Klein RS, Friedland GH, Moll B, Emeson EE, Spigland I, Steigbigel NH (1983) Immunodeficiency in female sexual partners of men with the acquired immunodeficiency syndrome. N Engl J Med 308:1181–1184

Harris LD, Tabb B, Sodora DL, Paiardini M, Klatt NR, Douek DC, Silvestri G, Muller-Trutwin M, Vasile-Pandrea I, Apetrei C, Hirsch V, Lifson J, Brenchley JM, Estes JD (2010) Down-regulation of robust acute type I interferon responses distinguishes nonpathogenic simian immunodeficiency virus (SIV) infection of natural hosts from pathogenic SIV infection of rhesus macaques. J Virol 84:7886–7891

Hart CE, Lennox JL, Pratt-Palmore M, Wright TC, Schinazi RF, Evans-Strickfaden T, Bush TJ, Schnell C, Conley LJ, Clancy KA, Ellerbrock TV (1999) Correlation of human immunodeficiency virus type 1 Rna levels in blood and the female genital tract. J Infect Dis 179:871–882

Hawkins C, Chalamilla G, Okuma J, Spiegelman D, Hertzmark E, Aris E, Ewald T, Mugusi F, Mtasiwa D, Fawzi W (2011) Sex differences in antiretroviral treatment outcomes among HIV-infected adults in an urban Tanzanian setting. AIDS 25:1189–1197

Hayashi J, Kishihara Y, Ueno K, Yamaji K, Kawakami Y, Furusyo N, Sawayama Y, Kashiwagi S (1998) Age-related response to interferon alfa treatment in women vs men with chronic hepatitis C virus infection. Arch Intern Med 158:177–181

Hayes RJ, Schulz KF, Plummer FA (1995) The cofactor effect of genital ulcers on the per-exposure risk of HIV transmission in sub-Saharan Africa. J Trop Med Hyg 98:1–8

Hazenberg MD, Otto SA, Van Benthem BH, Roos MT, Coutinho RA, Lange JM, Hamann D, Prins M, Miedema F (2003) Persistent immune activation in HIV-1 infection is associated with progression to Aids. AIDS 17:1881–1888

Heard I, Potard V, Costagliola D, Kazatchkine MD (2004) Contraceptive use in HIV-positive women. J Acquir Immune Defic Syndr 36:714–720

Heath SL, Tew JG, Tew JG, Szakal AK, Burton GF (1995) Follicular dendritic cells and human immunodeficiency virus infectivity. Nature 377:740–744

Heffron R, Donnell D, Rees H, Celum C, Mugo N, Were E, De Bruyn G, Nakku-Joloba E, Ngure K, Kiarie J, Coombs RW, Baeten JM (2012) Use of hormonal contraceptives and risk of HIV-1 transmission: a prospective cohort study. Lancet Infect Dis 12:19–26

Heffron R, Mugo N, Ngure K, Celum C, Donnell D, Were E, Rees H, Kiarie J, Baeten JM (2013) Hormonal contraceptive use and risk of HIV-1 disease progression. AIDS 27:261–267

Heggelund L, Muller F, Lien E, Yndestad A, Ueland T, Kristiansen KI, Espevik T, Aukrust P, Froland SS (2004) Increased expression of toll-like receptor 2 on monocytes in HIV infection: possible roles in inflammation and viral replication. Clin Infect Dis 39:264–269

Heikinheimo O, Lehtovirta P, Suni J, Paavonen J (2006) The levonorgestrel-releasing intrauterine system (Lng-Ius) in HIV-infected women–effects on bleeding patterns, ovarian function and genital shedding of HIV. Hum Reprod 21:2857–2861

Hel Z, Stringer E, Mestecky J (2010) Sex steroid hormones, hormonal contraception, and the immunobiology of human immunodeficiency virus-1 infection. Endocr Rev 31:79–97

Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, Hasemeier CM (1997) Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. Pediatrics 99: 505–512

Herman-Giddens ME, Wang L, Koch G (2001) Secondary sexual characteristics in boys: estimates from the national health and nutrition examination survey Iii, 1988–1994. Arch Pediatr Adolesc Med 155:1022–1028

Hessamfar-Bonarek M, Morlat P, Salmon D, Cacoub P, May T, Bonnet F, Rosenthal E, Costagliola D, Lewden C, Chene G (2010) Causes of death in HIV-infected women: persistent role of Aids. The 'Mortalite 2000 & 2005' Surveys (Anrs En19). Int J Epidemiol 39:135–146

Higgins JA, Hoffman S, Dworkin SL (2010) Rethinking gender, heterosexual men, and women's vulnerability to HIV/Aids. Am J Public Health 100:435–445

Hild-Petito S, Veazey RS, Larner JM, Reel JR, Blye RP (1998) Effects of two progestin-only contraceptives, Depo-Provera and Norplant-II, on the vaginal epithelium of rhesus monkeys. AIDS Res Hum Retroviruses 14(Suppl 1):S125–S130

Hira SK, Feldblum PJ, Kamanga J, Mukelabai G, Weir SS, Thomas JC (1997) Condom and nonoxynol-9 use and the incidence of HIV infection in serodiscordant couples in Zambia. Int J STD AIDS 8:243–250

Hira SK, Nkowane BM, Kamanga J, Wadhawan D, Kavindele D, Macuacua R, Mpoko G, Malek M, Cruess DF, Perine PL (1990) Epidemiology of human immunodeficiency virus in families in Lusaka, Zambia. J Acquir Immune Defic Syndr 3:83–86

Hladik F, Hope TJ (2009) HIV infection of the genital mucosa in women. Curr HIV/AIDS Rep 6: 20–28

Hladik F, Mcelrath MJ (2008) Setting the stage: host invasion by HIV. Nat Rev Immunol 8: 447–457

Hladik F, Sakchalathorn P, Ballweber L, Lentz G, Fialkow M, Eschenbach D, Mcelrath MJ (2007) Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. Immunity 26:257–270

Ho JE, Deeks SG, Hecht FM, Xie Y, Schnell A, Martin JN, Ganz P, Hsue PY (2010) Initiation of antiretroviral therapy at higher nadir Cd4+ T-cell counts is associated with reduced arterial stiffness in HIV-infected individuals. AIDS 24:1897–1905

Holmgren B, Da Silva Z, Larsen O, Vastrup P, Andersson S, Aaby P (2003) Dual infections with HIV-1, HIV-2 and Htlv-I are more common in older women than in men in Guinea-Bissau. AIDS 17:241–253

Holmgren B, Da Silva Z, Vastrup P, Larsen O, Andersson S, Ravn H, Aaby P (2007) Mortality associated with HIV-1, HIV-2, and Htlv-I single and dual infections in a middle-aged and older population in Guinea-Bissau. Retrovirology 4:85

Hu J, Gardner MB, Miller CJ (2000) Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells. J Virol 74:6087–6095

Hu J, Pope M, Brown C, O'doherty U, Miller CJ (1998) Immunophenotypic characterization of simian immunodeficiency virus-infected dendritic cells in cervix, vagina, and draining lymph nodes of rhesus monkeys. Lab Invest 78:435–451

Hubacher D, Liku J, Kiarie J, Rakwar J, Muiruri P, Omwenga J, Chen PL (2013) Effect of concurrent use of anti-retroviral therapy and levonorgestrel sub-dermal implant for contraception on Cd4 counts: a prospective cohort study in Kenya. J Int AIDS Soc 16:18448

Hughes GC, Thomas S, Li C, Kaja MK, Clark EA (2008) Cutting edge: progesterone regulates Ifn-alpha production by plasmacytoid dendritic cells. J Immunol 180:2029–2033

Hughes JP, Baeten JM, Lingappa JR, Magaret AS, Wald A, De Bruyn G, Kiarie J, Inambao M, Kilembe W, Farquhar C, Celum C (2012) Determinants of per-coital-act HIV-1 infectivity among African HIV-1-serodiscordant couples. J Infect Dis 205:358–365

Huijbregts RP, Helton ES, Michel KG, Sabbaj S, Richter HE, Goepfert PA, Hel Z (2013) Hormonal contraception and HIV-1 infection: medroxyprogesterone acetate suppresses innate and adaptive immune mechanisms. Endocrinology 154:1282–1295

Hulgan T, Shepherd BE, Raffanti SP, Fusco JS, Beckerman R, Barkanic G, Sterling TR (2007) Absolute count and percentage of Cd4+ lymphocytes are independent predictors of disease progression in HIV-infected persons initiating highly active antiretroviral therapy. J Infect Dis 195:425–431

Hunt PW, Deeks SG, Rodriguez B, Valdez H, Shade SB, Abrams DI, Kitahata MM, Krone M, Neilands TB, Brand RJ, Lederman MM, Martin JN (2003a) Continued Cd4 cell count increases in HIV-infected adults experiencing 4 years of viral suppression on antiretroviral therapy. AIDS 17:1907–1915

Hunt PW, Martin JN, Sinclair E, Bredt B, Hagos E, Lampiris H, Deeks SG (2003b) T cell activation is associated with lower Cd4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. J Infect Dis 187:1534–1543

Huthoff H, Towers GJ (2008) Restriction of retroviral replication by Apobec3G/F and Trim5alpha. Trends Microbiol 16:612–619

Hwang LY, Scott ME, Ma Y, Moscicki AB (2011) Higher levels of cervicovaginal inflammatory and regulatory cytokines and chemokines in healthy young women with immature cervical epithelium. J Reprod Immunol 88:66–71

Ildgruben AK, Sjoberg IM, Hammarstrom ML (2003) Influence of hormonal contraceptives on the immune cells and thickness of human vaginal epithelium. Obstet Gynecol 102:571–582

Intra M, Gentilini O, Brenelli F, Chagas EM, Veronesi U, Sandri MT (2005) Breast cancer among HIV-infected patients: the experience of the European Institute of Oncology. J Surg Oncol 91: 141–142

Ito A, Buenafe AC, Matejuk A, Zamora A, Silverman M, Dwyer J, Vandenbark AA, Offner H (2002) Estrogen inhibits systemic T cell expression of Tnf-alpha and recruitment of Tnf-alpha (+) T cells and macrophages into the Cns of mice developing experimental encephalomyelitis. Clin Immunol 102:275–282

Ito T, Kanzler H, Duramad O, Cao W, Liu YJ (2006) Specialization, kinetics, and repertoire of type 1 interferon responses by human plasmacytoid predendritic cells. Blood 107:2423–2431

Iversen AK, Larsen AR, Jensen T, Fugger L, Balslev U, Wahl S, Gerstoft J, Mullins JI, Skinhoj P (1998) Distinct determinants of human immunodeficiency virus type 1 Rna and Dna loads in vaginal and cervical secretions. J Infect Dis 177:1214–1220

Ivy W 3rd, Miles I, Le B, Paz-Bailey G (2013) Correlates of HIV Infection among African American Women from 20 Cities in the United States. Aids Behav

Iwasaki A (2010) Antiviral immune responses in the genital tract: clues for vaccines. Nat Rev Immunol 10:699–711

Jackson JB, Musoke P, Fleming T, Guay LA, Bagenda D, Allen M, Nakabiito C, Sherman J, Bakaki P, Owor M, Ducar C, Deseyve M, Mwatha A, Emel L, Duefield C, Mirochnick M, Fowler MG, Mofenson L, Miotti P, Gigliotti M, Bray D, Mmiro F (2003) Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: 18-month follow-up of the HIVnet 012 randomised trial. Lancet 362:859–868

Jacobson DL, Peralta L, Farmer M, Graham NM, Gaydos C, Zenilman J (2000) Relationship of hormonal contraception and cervical ectopy as measured by computerized planimetry to chlamydial infection in adolescents. Sex Transm Dis 27:313–319

Jacquelin B, Mayau V, Targat B, Liovat AS, Kunkel D, Petitjean G, Dillies MA, Roques P, Butor C, Silvestri G, Giavedoni LD, Lebon P, Barre-Sinoussi F, Benecke A, Muller-Trutwin MC (2009) Nonpathogenic SIV infection of African green monkeys induces a strong but rapidly controlled type I Ifn response. J Clin Invest 119:3544–3555

Jaffar S, Grant AD, Whitworth J, Smith PG, Whittle H (2004) The natural history of HIV-1 and HIV-2 infections in adults in Africa: a literature review. Bull World Health Organ 82:462–469

Jain R, Muralidhar S (2011) Contraceptive methods: needs, options and utilization. J Obstet Gynaecol India 61:626–634

Jarrin I, Geskus R, Bhaskaran K, Prins M, Perez-Hoyos S, Muga R, Hernandez-Aguado I, Meyer L, Porter K, Del Amo J (2008) Gender differences in HIV progression to Aids and death in industrialized countries: slower disease progression following HIV seroconversion in women. Am J Epidemiol 168:532–540

Jaspan HB, Liebenberg L, Hanekom W, Burgers W, Coetzee D, Williamson AL, Little F, Myer L, Coombs RW, Sodora D, Passmore JA (2011) Immune activation in the female genital tract during HIV infection predicts mucosal Cd4 depletion and HIV shedding. J Infect Dis 204: 1550–1556

Jennes W, Evertse D, Borget MY, Vuylsteke B, Maurice C, Nkengasong JN, Kestens L (2006) Suppressed cellular alloimmune responses in HIV-exposed seronegative female sex workers. Clin Exp Immunol 143:435–444

Jennes W, Sawadogo S, Koblavi-Deme S, Vuylsteke B, Maurice C, Roels TH, Chorba T, Nkengasong JN, Kestens L (2003) Cellular human immunodeficiency virus (HIV)-protective factors: a comparison of HIV-exposed seronegative female sex workers and female blood donors in Abidjan, Cote d'Ivoire. J Infect Dis 187:206–214

Jewkes RK, Dunkle K, Nduna M, Shai N (2010) Intimate partner violence, relationship power inequity, and incidence of HIV infection in young women in South Africa: a cohort study. Lancet 376:41–48

Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, Landay A, Martin J, Sinclair E, Asher AI, Deeks SG, Douek DC, Brenchley JM (2009) Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. J Infect Dis 199:1177–1185

Jie C, Tulpule A, Zheng T, Masood R, Espina B, Gill PS (1997) Treatment of epidemic (Aids-related) Kaposi's sarcoma. Curr Opin Oncol 9:433–439

Johnson AM, Petherick A, Davidson SJ, Brettle R, Hooker M, Howard L, Mclean KA, Osborne LE, Robertson R, Sonnex C et al (1989) Transmission of HIV to heterosexual partners of infected men and women. AIDS 3:367–372

Johnson LF, Lewis DA (2008) The effect of genital tract infections on HIV-1 shedding in the genital tract: a systematic review and meta-analysis. Sex Transm Dis 35:946–959

Junghans C, Low N, Chan P, Witschi A, Vernazza P, Egger M (1999) Uniform risk of clinical progression despite differences in utilization of highly active antiretroviral therapy: Swiss HIV Cohort Study. AIDS 13:2547–2554

Kacani L, Prodinger WM, Sprinzl GM, Schwendinger MG, Spruth M, Stoiber H, Dopper S, Steinhuber S, Steindl F, Dierich MP (2000) Detachment of human immunodeficiency virus type 1 from germinal centers by blocking complement receptor type 2. J Virol 74:7997–8002

Kali PB, Gray GE, Violari A, Chaisson RE, Mcintyre JA, Martinson NA (2006) Combining PMTCT with active case finding for tuberculosis. J Acquir Immune Defic Syndr 42:379–381

Kampinga GA, Simonon A, Van De Perre P, Karita E, Msellati P, Goudsmit J (1997) Primary infections with HIV-1 of women and their offspring in Rwanda: findings of heterogeneity at seroconversion, coinfection, and recombinants of HIV-1 subtypes A and C. Virology 227: 63–76

Kapiga SH, Lyamuya EF, Lwihula GK, Hunter DJ (1998) The incidence of HIV infection among women using family planning methods in Dar es Salaam, Tanzania. AIDS 12:75–84

Karpati AM, Rubin CH, Kieszak SM, Marcus M, Troiano RP (2002) Stature and pubertal stage assessment in American boys: the 1988–1994 Third National Health and Nutrition Examination Survey. J Adolesc Health 30:205–212

Katzenstein DA, Hammer SM, Hughes MD, Gundacker H, Jackson JB, Fiscus S, Rasheed S, Elbeik T, Reichman R, Japour A, Merigan TC, Hirsch MS (1996) The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 Cd4 cells per cubic millimeter. Aids Clinical Trials Group Study 175 Virology Study Team. N Engl J Med 335:1091–1098

Kaul R, Pettengell C, Sheth PM, Sunderji S, Biringer A, Macdonald K, Walmsley S, Rebbapragada A (2008) The genital tract immune milieu: an important determinant of HIV susceptibility and secondary transmission. J Reprod Immunol 77:32–40

Kaushic C, Ashkar AA, Reid LA, Rosenthal KL (2003) Progesterone increases susceptibility and decreases immune responses to genital herpes infection. J Virol 77:4558–4565

Kaushic C, Ferreira VH, Kafka JK, Nazli A (2010) HIV infection in the female genital tract: discrete influence of the local mucosal microenvironment. Am J Reprod Immunol 63:566–575

Kaushic C, Murdin AD, Underdown BJ, Wira CR (1998) Chlamydia trachomatis infection in the female reproductive tract of the rat: influence of progesterone on infectivity and immune response. Infect Immun 66:893–898

Kaushic C, Roth KL, Anipindi V, Xiu F (2011) Increased prevalence of sexually transmitted viral infections in women: the role of female sex hormones in regulating susceptibility and immune responses. J Reprod Immunol 88:204–209

Keele BF, Tazi L, Gartner S, Liu Y, Burgon TB, Estes JD, Thacker TC, Crandall KA, Mcarthur JC, Burton GF (2008) Characterization of the follicular dendritic cell reservoir of human immunodeficiency virus type 1. J Virol 82:5548–5561

Keller ET, Zhang J, Yao Z, Qi Y (2001) The impact of chronic estrogen deprivation on immunologic parameters in the ovariectomized rhesus monkey (Macaca mulatta) model of menopause. J Reprod Immunol 50:41–55

Kiddugavu M, Makumbi F, Wawer MJ, Serwadda D, Sewankambo NK, Wabwire-Mangen F, Lutalo T, Meehan M, Xianbin, Gray RH (2003) Hormonal contraceptive use and HIV-1 infection in a population-based cohort in Rakai, Uganda. AIDS 17:233–240

Kigozi G, Wawer M, Ssettuba A, Kagaayi J, Nalugoda F, Watya S, Mangen FW, Kiwanuka N, Bacon MC, Lutalo T, Serwadda D, Gray RH (2009) Foreskin surface area and HIV acquisition in Rakai, Uganda (size matters). AIDS 23:2209–2213

Kilian AH, Gregson S, Ndyanabangi B, Walusaga K, Kipp W, Sahlmuller G, Garnett GP, Asiimwe-Okiror G, Kabagambe G, Weis P, Von Sonnenburg F (1999) Reductions in risk behaviour provide the most consistent explanation for declining HIV-1 prevalence in Uganda. AIDS 13:391–398

Kipp W, Alibhai A, Saunders LD, Senthilselvan A, Kaler A, Konde-Lule J, Okech-Ojony J, Rubaale T (2010) Gender differences in antiretroviral treatment outcomes of HIV patients in rural Uganda. Aids Care 22:271–278

Kirkham CM, Lobb DJ (1998) The British Columbia Positive Women's Survey: a detailed profile of 110 HIV-infected women. CMAJ 158:317–323

Kissinger P, Amedee A, Clark RA, Dumestre J, Theall KP, Myers L, Hagensee ME, Farley TA, Martin DH (2009) Trichomonas vaginalis treatment reduces vaginal HIV-1 shedding. Sex Transm Dis 36:11–16

Kissinger P, Secor WE, Leichliter JS, Clark RA, Schmidt N, Curtin E, Martin DH (2008) Early repeated infections with Trichomonas vaginalis among HIV-positive and HIV-negative women. Clin Infect Dis 46:994–999

Klausner JD, Serenata C, O'Bra H, Mattson CL, Brown JW, Wilson M, Mbengashe T, Goldman TM (2011) Scale-up and continuation of antiretroviral therapy in South African treatment programs, 2005–2009. J Acquir Immune Defic Syndr 56:292–295

Klebanoff SJ, Coombs RW (1991) Viricidal effect of Lactobacillus acidophilus on human immunodeficiency virus type 1: possible role in heterosexual transmission. J Exp Med 174: 289–292

Kontula K, Paavonen T, Luukkainen T, Andersson LC (1983) Binding of progestins to the glucocorticoid receptor. Correlation to their glucocorticoid-like effects on in vitro functions of human mononuclear leukocytes. Biochem Pharmacol 32:1511–1518

Koskinas J, Merkouraki P, Manesis E, Hadziyannis S (2002) Assessment of depression in patients with chronic hepatitis: effect of interferon treatment. Dig Dis 20:284–288

Koubovec D, Ronacher K, Stubsrud E, Louw A, Hapgood JP (2005) Synthetic progestins used in Hrt have different glucocorticoid agonist properties. Mol Cell Endocrinol 242:23–32

Kouyoumdjian FG, Calzavara LM, Bondy SJ, O'campo P, Serwadda D, Nalugoda F, Kagaayi J, Kigozi G, Wawer M, Gray R (2013) Intimate partner violence is associated with incident HIV infection in women in Uganda. AIDS 27:1331–1338

Kovacs A, Wasserman SS, Burns D, Wright DJ, Cohn J, Landay A, Weber K, Cohen M, Levine A, Minkoff H, Miotti P, Palefsky J, Young M, Reichelderfer P (2001) Determinants of HIV-1 shedding in the genital tract of women. Lancet 358:1593–1601

Krantz EM, Hullsiek KH, Okulicz JF, Weintrob AC, Agan BK, Crum-Cianflone NF, Ganesan A, Ferguson TM, Hale BR (2011) Elevated Cd8 counts during Haart are associated with HIV virologic treatment failure. J Acquir Immune Defic Syndr 57:396–403

Krug EG, Mercy JA, Dahlberg LL, Zwi AB (2002) The world report on violence and health. Lancet 360:1083–1088

Kutteh WH, Prince SJ, Hammond KR, Kutteh CC, Mestecky J (1996) Variations in immunoglobulins and IgA subclasses of human uterine cervical secretions around the time of ovulation. Clin Exp Immunol 104:538–542

La Ferla L, Pinzone MR, Nunnari G, Martellotta F, Lleshi A, Tirelli U, De Paoli P, Berretta M, Cacopardo B (2013) Kaposi' s sarcoma in HIV-positive patients: the state of art in the Haart-era. Eur Rev Med Pharmacol Sci 17:2354–2365

Laga M, Alary M, Nzila N, Manoka AT, Tuliza M, Behets F, Goeman J, St Louis M, Piot P (1994) Condom promotion, sexually transmitted diseases treatment, and declining incidence of HIV-1 infection in female Zairian sex workers. Lancet 344:246–248

Laga M, Manoka A, Kivuvu M, Malele B, Tuliza M, Nzila N, Goeman J, Behets F, Batter V, Alary M et al (1993) Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. AIDS 7:95–102

Larsen O, Da Silva Z, Sandstrom A, Andersen PK, Andersson S, Poulsen AG, Melbye M, Dias F, Naucler A, Aaby P (1998) Declining HIV-2 prevalence and incidence among men in a community study from Guinea-Bissau. AIDS 12:1707–1714

Laskarin G, Strbo N, Sotosek V, Rukavina D, Faust Z, Szekeres-Bartho J, Podack ER (1999) Progesterone directly and indirectly affects perforin expression in cytolytic cells. Am J Reprod Immunol 42:312–320

Lassoued K, Clauvel JP, Fegueux S, Matheron S, Gorin I, Oksenhendler E (1991) Aids-associated Kaposi's sarcoma in female patients. AIDS 5:877–880

Lavreys L, Baeten JM, Chohan V, Mcclelland RS, Hassan WM, Richardson BA, Mandaliya K, Ndinya-Achola JO, Overbaugh J (2006) Higher set point plasma viral load and more-severe acute HIV type 1 (HIV-1) illness predict mortality among high-risk HIV-1-infected African women. Clin Infect Dis 42:1333–1339

Lavreys L, Baeten JM, Kreiss JK, Richardson BA, Chohan BH, Hassan W, Panteleeff DD, Mandaliya K, Ndinya-Achola JO, Overbaugh J (2004a) Injectable contraceptive use and genital ulcer disease during the early phase of HIV-1 infection increase plasma virus load in women. J Infect Dis 189:303–311

Lavreys L, Baeten JM, Martin HL Jr, Overbaugh J, Mandaliya K, Ndinya-Achola J, Kreiss JK (2004b) Hormonal contraception and risk of HIV-1 acquisition: results of a 10-year prospective study. AIDS 18:695–697

Lavreys L, Chohan V, Overbaugh J, Hassan W, Mcclelland RS, Kreiss J, Mandaliya K, Ndinya-Achola J, Baeten JM (2004c) Hormonal contraception and risk of cervical infections among HIV-1-seropositive Kenyan women. AIDS 18:2179–2184

Lavreys L, Rakwar JP, Thompson ML, Jackson DJ, Mandaliya K, Chohan BH, Bwayo JJ, Ndinya-Achola JO, Kreiss JK (1999) Effect of circumcision on incidence of human immunodeficiency virus type 1 and other sexually transmitted diseases: a prospective cohort study of trucking company employees in Kenya. J Infect Dis 180:330–336

Lawn SD, Butera ST, Folks TM (2001) Contribution of immune activation to the pathogenesis and transmission of human immunodeficiency virus type 1 infection. Clin Microbiol Rev 14: 753–777, table of contents

Lazzarin A, Saracco A, Musicco M, Nicolosi A (1991) Man-to-woman sexual transmission of the human immunodeficiency virus. Risk factors related to sexual behavior, man's infectiousness, and woman's susceptibility. Italian Study Group on HIV Heterosexual Transmission. Arch Intern Med 151:2411–2416

Leclerc PM, Dubois-Colas N, Garenne M (2008) Hormonal contraception and HIV prevalence in four African countries. Contraception 77:371–376

Lee MN, Roy M, Ong SE, Mertins P, Villani AC, Li W, Dotiwala F, Sen J, Doench JG, Orzalli MH, Kramnik I, Knipe DM, Lieberman J, Carr SA, Hacohen N (2013) Identification of regulators of the innate immune response to cytosolic Dna and retroviral infection by an integrative approach. Nat Immunol 14:179–185

Lekakis J, Ikonomidis I (2010) Cardiovascular complications of Aids. Curr Opin Crit Care 16: 408–412

Lemly DC, Shepherd BE, Hulgan T, Rebeiro P, Stinnette S, Blackwell RB, Bebawy S, Kheshti A, Sterling TR, Raffanti SP (2009) Race and sex differences in antiretroviral therapy use and mortality among HIV-infected persons in care. J Infect Dis 199:991–998

Lemp GF, Hirozawa AM, Cohen JB, Derish PA, Mckinney KC, Hernandez SR (1992) Survival for women and men with Aids. J Infect Dis 166:74–79

Lester RT, Yao XD, Ball TB, Mckinnon LR, Kaul R, Wachihi C, Jaoko W, Plummer FA, Rosenthal KL (2008) Toll-like receptor expression and responsiveness are increased in viraemic HIV-1 infection. AIDS 22:685–694

Levinson P, Kaul R, Kimani J, Ngugi E, Moses S, Macdonald KS, Broliden K, Hirbod T (2009) Levels of innate immune factors in genital fluids: association of alpha defensins and Ll-37 with genital infections and increased HIV acquisition. AIDS 23:309–317

Lewden C, May T, Rosenthal E, Burty C, Bonnet F, Costagliola D, Jougla E, Semaille C, Morlat P, Salmon D, Cacoub P, Chene G (2008) Changes in causes of death among adults infected by HIV between 2000 and 2005: The "Mortalite 2000 and 2005" surveys (Anrs En19 and Mortavic). J Acquir Immune Defic Syndr 48:590–598

Lewden C, Salmon D, Morlat P, Bevilacqua S, Jougla E, Bonnet F, Heripret L, Costagliola D, May T, Chene G (2005) Causes of death among human immunodeficiency virus (HIV)-infected adults in the era of potent antiretroviral therapy: emerging role of hepatitis and cancers, persistent role of Aids. Int J Epidemiol 34:121–130

Li Q, Estes JD, Schlievert PM, Duan L, Brosnahan AJ, Southern PJ, Reilly CS, Peterson ML, Schultz-Darken N, Brunner KG, Nephew KR, Pambuccian S, Lifson JD, Carlis JV, Haase AT (2009) Glycerol monolaurate prevents mucosal SIV transmission. Nature 458:1034–1038

Lichtenstein KA, Armon C, Buchacz K, Chmiel JS, Buckner K, Tedaldi EM, Wood K, Holmberg SD, Brooks JT (2010) Low Cd4+ T cell count is a risk factor for cardiovascular disease events in the HIV outpatient study. Clin Infect Dis 51:435–447

Lipsitz JD, Williams JB, Rabkin JG, Remien RH, Bradbury M, El Sadr W, Goetz R, Sorrell S, Gorman JM (1994) Psychopathology in male and female intravenous drug users with and without HIV infection. Am J Psychiatry 151:1662–1668

Long EM, Martin HL Jr, Kreiss JK, Rainwater SM, Lavreys L, Jackson DJ, Rakwar J, Mandaliya K, Overbaugh J (2000) Gender differences in HIV-1 diversity at time of infection. Nat Med 6:71–75

Lopman B, Lewis J, Nyamukapa C, Mushati P, Chandiwana S, Gregson S (2007) HIV incidence and poverty in Manicaland, Zimbabwe: is HIV becoming a disease of the poor? AIDS 21(Suppl 7):S57–S66

Loupa CV, Rodriguez B, Mccomsey G, Gripshover B, Salata RA, Valdez H, Lisgaris MV, Fulton SA, Lederman MM (2006) Gender differences in human immunodeficiency virus (HIV) RNA and CD4 cell counts among new entrants to HIV care. Clin Microbiol Infect 12: 389–391

Loutfy MR, Wu W, Letchumanan M, Bondy L, Antoniou T, Margolese S, Zhang Y, Rueda S, Mcgee F, Peck R, Binder L, Allard P, Rourke SB, Rochon PA (2013) Systematic review of HIV transmission between heterosexual serodiscordant couples where the HIV-positive partner is fully suppressed on antiretroviral therapy. PLoS One 8:e55747

Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, De Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E,

Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL et al (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2095–2128

Lu FX, Ma Z, Rourke T, Srinivasan S, Mcchesney M, Miller CJ (1999) Immunoglobulin concentrations and antigen-specific antibody levels in cervicovaginal lavages of rhesus macaques are influenced by the stage of the menstrual cycle. Infect Immun 67:6321–6328

Lu FX, Abel K, Ma Z, Rourke T, Lu D, Torten J, Mcchesney M, Miller CJ (2002) The strength of B cell immunity in female rhesus macaques is controlled by Cd8+ T cells under the influence of ovarian steroid hormones. Clin Exp Immunol 128:10–20

Lu FX, Ma Z, Moser S, Evans TG, Miller CJ (2003) Effects of ovarian steroids on immunoglobulin-secreting cell function in healthy women. Clin Diagn Lab Immunol 10: 944–949

Lucia MB, Anu R, Handley M, Gillet JP, Wu CP, De Donatis GM, Cauda R, Gottesman MM (2011) Exposure to HIV-protease inhibitors selects for increased expression of P-glycoprotein (Abcb1) in Kaposi's sarcoma cells. Br J Cancer 105:513–522

Lusher JM, Operskalski EA, Aledort LM, Dietrich SL, Gjerset GF, Hilgartner MW, Koerper MA, Pegelow CH, Lee H, Mosley JW (1991) Risk of human immunodeficiency virus type 1 infection among sexual and nonsexual household contacts of persons with congenital clotting disorders. Pediatrics 88:242–249

Ly KN, Xing J, Klevens RM, Jiles RB, Ward JW, Holmberg SD (2012) The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. Ann Intern Med 156:271–278

Macias J, Berenguer J, Japon MA, Giron JA, Rivero A, Lopez-Cortes LF, Moreno A, Gonzalez-Serrano M, Iribarren JA, Ortega E, Miralles P, Mira JA, Pineda JA (2009) Fast fibrosis progression between repeated liver biopsies in patients coinfected with human immunodeficiency virus/hepatitis C virus. Hepatology 50:1056–1063

Macpherson AJ, Uhr T (2004a) Compartmentalization of the mucosal immune responses to commensal intestinal bacteria. Ann NY Acad Sci 1029:36–43

Macpherson AJ, Uhr T (2004b) Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science 303:1662–1665

Magnus M, Clark R, Myers L, Farley T, Kissinger PJ (2003) Trichomonas vaginalis among HIV-Infected women: are immune status or protease inhibitor use associated with subsequent T. vaginalis positivity? Sex Transm Dis 30:839–843

Mahoney EM, Donfield SM, Howard C, Kaufman F, Gertner JM (1999) HIV-associated immune dysfunction and delayed pubertal development in a cohort of young hemophiliacs. Hemophilia Growth and Development Study. J Acquir Immune Defic Syndr 21:333–337

Maini MK, Gilson RJ, Chavda N, Gill S, Fakoya A, Ross EJ, Phillips AN, Weller IV (1996) Reference ranges and sources of variability of Cd4 counts in HIV-seronegative women and men. Genitourin Med 72:27–31

Majaliwa ES, Mohn A, Chiarelli F (2009) Growth and puberty in children with HIV infection. J Endocrinol Invest 32:85–90

Manches O, Munn D, Fallahi A, Lifson J, Chaperot L, Plumas J, Bhardwaj N (2008) HIV-activated human plasmacytoid DCS induce TREGS through an indoleamine 2,3-dioxygenase-dependent mechanism. J Clin Invest 118:3431–3439

Marcelin AG, Tubiana R, Lambert-Niclot S, Lefebvre G, Dominguez S, Bonmarchand M, Vauthier-Brouzes D, Marguet F, Mousset-Simeon N, Peytavin G, Poirot C (2008) Detection of HIV-1 Rna in seminal plasma samples from treated patients with undetectable HIV-1 RNA in blood plasma. AIDS 22:1677–1679

Marlink R, Kanki P, Thior I, Travers K, Eisen G, Siby T, Traore I, Hsieh CC, Dia MC, Gueye EH et al (1994) Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science 265:1587–1590

Martin HL Jr, Nyange PM, Richardson BA, Lavreys L, Mandaliya K, Jackson DJ, Ndinya-Achola JO, Kreiss J (1998a) Hormonal contraception, sexually transmitted diseases, and risk of heterosexual transmission of human immunodeficiency virus type 1. J Infect Dis 178: 1053–1059

Martin HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B, Mandaliya K, Ndinya-Achola JO, Bwayo J, Kreiss J (1999) Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infect Dis 180:1863–1868

Martin RM, Biswas PN, Freemantle SN, Pearce GL, Mann RD (1998b) Age and sex distribution of suspected adverse drug reactions to newly marketed drugs in general practice in England: analysis of 48 cohort studies. Br J Clin Pharmacol 46:505–511

Martinson JA, Roman-Gonzalez A, Tenorio AR, Montoya CJ, Gichinga CN, Rugeles MT, Tomai M, Krieg AM, Ghanekar S, Baum LL, Landay AL (2007) Dendritic cells from HIV-1 infected individuals are less responsive to toll-like receptor (Tlr) ligands. Cell Immunol 250: 75–84

Martro E, Esteve A, Schulz TF, Sheldon J, Gambus G, Munoz R, Whitby D, Casabona J (2007) Risk factors for human Herpesvirus 8 infection and Aids-associated Kaposi's sarcoma among men who have sex with men in a European multicentre study. Int J Cancer 120:1129–1135

Marx PA, Spira AI, Gettie A, Dailey PJ, Veazey RS, Lackner AA, Mahoney CJ, Miller CJ, Claypool LE, Ho DD, Alexander NJ (1996) Progesterone implants enhance SIV vaginal transmission and early virus load. Nat Med 2:1084–1089

Maskew M, Macphail AP, Whitby D, Egger M, Wallis CL, Fox MP (2011) Prevalence and predictors of kaposi sarcoma herpes virus seropositivity: a cross-sectional analysis of HIV-infected adults initiating Art in Johannesburg, South Africa. Infect Agent Cancer 6:22

Massad LS, Evans CT, Wilson TE, Golub ET, Sanchez-Keeland L, Minkoff H, Weber K, Watts DH (2007) Contraceptive use among U.S. women with HIV. J Womens Health (Larchmt) 16: 657–666

Mastro TD, De Vincenzi I (1996) Probabilities of sexual HIV-1 transmission. AIDS 10(Suppl A): S75–S82

Mati JK, Hunter DJ, Maggwa BN, Tukei PM (1995) Contraceptive use and the risk of HIV infection in Nairobi, Kenya. Int J Gynaecol Obstet 48:61–67

Mauck CK, Callahan MM, Baker J, Arbogast K, Veazey R, Stock R, Pan Z, Morrison CS, Chen-Mok M, Archer DF, Gabelnick HL (1999) The effect of one injection of Depo-Provera on the human vaginal epithelium and cervical ectopy. Contraception 60:15–24

Mavedzenge SN, Pol BV, Cheng H, Montgomery ET, Blanchard K, De Bruyn G, Ramjee G, Straten A (2010) Epidemiological synergy of Trichomonas vaginalis and HIV in Zimbabwean and South African women. Sex Transm Dis 37:460–466

Mayer KH, Venkatesh KK (2011) Interactions of HIV, other sexually transmitted diseases, and genital tract inflammation facilitating local pathogen transmission and acquisition. Am J Reprod Immunol 65:308–316

Mcclelland RS, Sangare L, Hassan WM, Lavreys L, Mandaliya K, Kiarie J, Ndinya-Achola J, Jaoko W, Baeten JM (2007) Infection with Trichomonas vaginalis increases the risk of HIV-1 acquisition. J Infect Dis 195:698–702

Mccoy SI, Zheng W, Montgomery ET, Blanchard K, Van Der Straten A, De Bruyn G, Padian NS (2013) Oral and injectable contraception use and risk of HIV acquisition among women in sub-Saharan Africa. AIDS 27:1001–1009

Mcgarvey ME, Tulpule A, Cai J, Zheng T, Masood R, Espina B, Arora N, Smith DL, Gill PS (1998) Emerging treatments for epidemic (Aids-related) Kaposi's sarcoma. Curr Opin Oncol 10:413–421

Mckinnon LR, Nyanga B, Chege D, Izulla P, Kimani M, Huibner S, Gelmon L, Block KE, Cicala C, Anzala AO, Arthos J, Kimani J, Kaul R (2011) Characterization of a human cervical Cd4+ T cell subset coexpressing multiple markers of HIV susceptibility. J Immunol 187: 6032–6042

Mclaren PJ, Ball TB, Wachihi C, Jaoko W, Kelvin DJ, Danesh A, Kimani J, Plummer FA, Fowke KR (2010) HIV-exposed seronegative commercial sex workers show a quiescent phenotype in the Cd4+ T cell compartment and reduced expression of HIV-dependent host factors. J Infect Dis 202(Suppl 3):S339–S344

Mcnearney T, Hornickova Z, Markham R, Birdwell A, Arens M, Saah A, Ratner L (1992) Relationship of human immunodeficiency virus type 1 sequence heterogeneity to stage of disease. Proc Natl Acad Sci U S A 89:10247–10251

Meditz AL, Borok M, Mawhinney S, Gudza I, Ndemera B, Gwanzura L, Campbell TB (2007) Gender differences in Aids-associated Kaposi sarcoma in Harare, Zimbabwe. J Acquir Immune Defic Syndr 44:306–308

Meier A, Chang JJ, Chan ES, Pollard RB, Sidhu HK, Kulkarni S, Wen TF, Lindsay RJ, Orellana L, Mildvan D, Bazner S, Streeck H, Alter G, Lifson JD, Carrington M, Bosch RJ, Robbins GK, Altfeld M (2009) Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. Nat Med 15:955–959

Melnick SL, Sherer R, Louis TA, Hillman D, Rodriguez EM, Lackman C, Capps L, Brown LS Jr, Carlyn M, Korvick JA et al (1994) Survival and disease progression according to gender of patients with HIV infection. The Terry Beirn Community Programs for Clinical Research on Aids. JAMA 272:1915–1921

Merad M, Romani N, Randolph G (2008) Langerhans cells at the interface of medicine, science, and industry. J Invest Dermatol 128:251–255

Micallef JM, Kaldor JM, Dore GJ (2006) Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat 13:34–41

Michael CW, Esfahani FM (1997) Pregnancy-related changes: a retrospective review of 278 cervical smears. Diagn Cytopathol 17:99–107

Michelo C, Sandoy IF, Fylkesnes K (2006) Marked HIV prevalence declines in higher educated young people: evidence from population-based surveys (1995-2003) in Zambia. AIDS 20: 1031–1038

Miller CJ, Li Q, Abel K, Kim EY, Ma ZM, Wietgrefe S, La Franco-Scheuch L, Compton L, Duan L, Shore MD, Zupancic M, Busch M, Carlis J, Wolinsky S, Haase AT (2005a) Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. J Virol 79:9217–9227

Miller K, Corcoran C, Armstrong C, Caramelli K, Anderson E, Cotton D, Basgoz N, Hirschhorn L, Tuomala R, Schoenfeld D, Daugherty C, Mazer N, Grinspoon S (1998) Transdermal testosterone administration in women with acquired immunodeficiency syndrome wasting: a pilot study. J Clin Endocrinol Metab 83:2717–2725

Miller L, Patton DL, Meier A, Thwin SS, Hooton TM, Eschenbach DA (2000) Depomedroxyprogesterone-induced hypoestrogenism and changes in vaginal flora and epithelium. Obstet Gynecol 96:431–439

Miller SA, Santoro N, Lo Y, Howard AA, Arnsten JH, Floris-Moore M, Moskaleva G, Schoenbaum EE (2005b) Menopause symptoms in HIV-infected and drug-using women. Menopause 12:348–356

Mills EJ, Bakanda C, Birungi J, Chan K, Hogg RS, Ford N, Nachega JB, Cooper CL (2011) Male gender predicts mortality in a large cohort of patients receiving antiretroviral therapy in Uganda. J Int AIDS Soc 14:52

Mingjia L, Short R (2002) How oestrogen or progesterone might change a woman's susceptibility to HIV-1 infection. Aust N Z J Obstet Gynaecol 42:472–475

Minkoff HL, Eisenberger-Matityahu D, Feldman J, Burk R, Clarke L (1999) Prevalence and incidence of gynecologic disorders among women infected with human immunodeficiency virus. Am J Obstet Gynecol 180:824–836

Mir KD, Gasper MA, Sundaravaradan V, Sodora DL (2011) SIV infection in natural hosts: resolution of immune activation during the acute-to-chronic transition phase. Microbes Infect 13:14–24

Mishra V, Assche SB, Greener R, Vaessen M, Hong R, Ghys PD, Boerma JT, Van Assche A, Khan S, Rutstein S (2007) HIV infection does not disproportionately affect the poorer in sub-Saharan Africa. AIDS 21(Suppl 7):S17–S28

Mock PA, Shaffer N, Bhadrakom C, Siriwasin W, Chotpitayasunondh T, Chearskul S, Young NL, Roongpisuthipong A, Chinayon P, Kalish ML, Parekh B, Mastro TD (1999) Maternal viral load and timing of mother-to-child HIV transmission, Bangkok. Thailand. Bangkok Collaborative Perinatal HIV Transmission Study Group. AIDS 13:407–414

Mocroft A, Gill MJ, Davidson W, Phillips AN (2000) Are there gender differences in starting protease inhibitors, Haart, and disease progression despite equal access to care? J Acquir Immune Defic Syndr 24:475–482

Mocroft A, Madge S, Johnson AM, Lazzarin A, Clumeck N, Goebel FD, Viard JP, Gatell J, Blaxhult A, Lundgren JD (1999) A comparison of exposure groups in the Eurosida study: starting highly active antiretroviral therapy (Haart), response to Haart, and survival. J Acquir Immune Defic Syndr 22:369–378

Molander U, Milsom I, Ekelund P, Mellstrom D, Eriksson O (1990) Effect of oral oestriol on vaginal flora and cytology and urogenital symptoms in the post-menopause. Maturitas 12: 113–120

Money DM, Arikan YY, Remple V, Sherlock C, Craib K, Birch P, Burdge DR (2003) Genital tract and plasma human immunodeficiency virus viral load throughout the menstrual cycle in women who are infected with ovulatory human immunodeficiency virus. Am J Obstet Gynecol 188:122–128

Monroe KM, Yang Z, Johnson JR, Geng X, Doitsh G, Krogan NJ, Greene WC (2014) Ifi16 DNA sensor is required for death of lymphoid Cd4 T cells abortively infected with HIV. Science 343:428–432

Moore AL, Kirk O, Johnson AM, Katlama C, Blaxhult A, Dietrich M, Colebunders R, Chiesi A, Lungren JD, Phillips AN (2003) Virologic, immunologic, and clinical response to highly active antiretroviral therapy: the gender issue revisited. J Acquir Immune Defic Syndr 32:452–461

Moore AL, Mocroft A, Madge S, Devereux H, Wilson D, Phillips AN, Johnson M (2001) Gender differences in virologic response to treatment in an HIV-positive population: a cohort study. J Acquir Immune Defic Syndr 26:159–163

Moore AL, Sabin CA, Johnson MA, Phillips AN (2002) Gender and clinical outcomes after starting highly active antiretroviral treatment: a cohort study. J Acquir Immune Defic Syndr 29:197–202

Moore J, Schuman P, Schoenbaum E, Boland B, Solomon L, Smith D (1999) Severe adverse life events and depressive symptoms among women with, or at risk for, HIV infection in four cities in the United States of America. AIDS 13:2459–2468

Moore RD, Hidalgo J, Sugland BW, Chaisson RE (1991) Zidovudine and the natural history of the acquired immunodeficiency syndrome. N Engl J Med 324:1412–1416

Mor G, Sapi E, Abrahams VM, Rutherford T, Song J, Hao XY, Muzaffar S, Kohen F (2003) Interaction of the estrogen receptors with the Fas ligand promoter in human monocytes. J Immunol 170:114–122

Morrison CS, Bright P, Wong EL, Kwok C, Yacobson I, Gaydos CA, Tucker HT, Blumenthal PD (2004) Hormonal contraceptive use, cervical ectopy, and the acquisition of cervical infections. Sex Transm Dis 31:561–567

Morrison CS, Chen PL, Kwok C, Richardson BA, Chipato T, Mugerwa R, Byamugisha J, Padian N, Celentano DD, Salata RA (2010) Hormonal contraception and HIV acquisition: reanalysis using marginal structural modeling. AIDS 24:1778–1781

Morrison CS, Nanda K (2012) Hormonal contraception and HIV: an unanswered question. Lancet Infect Dis 12:2–3

Morrison CS, Richardson BA, Mmiro F, Chipato T, Celentano DD, Luoto J, Mugerwa R, Padian N, Rugpao S, Brown JM, Cornelisse P, Salata RA (2007a) Hormonal contraception and the risk of HIV acquisition. AIDS 21:85–95

Morrison CS, Skoler-Karpoff S, Kwok C, Chen PL, Van De Wijgert J, Gehret-Plagianos M, Patel S, Ahmed K, Ramjee G, Friedland B, Lahteenmaki P (2012) Hormonal contraception and the risk of HIV acquisition among women in South Africa. AIDS 26:497–504

Morrison CS, Wang J, Van Der Pol B, Padian N, Salata RA, Richardson BA (2007b) Pregnancy and the risk of HIV-1 acquisition among women in Uganda and Zimbabwe. AIDS 21: 1027–1034

Mosam A, Shaik F, Uldrick TS, Esterhuizen T, Friedland GH, Scadden DT, Aboobaker J, Coovadia HM (2012) A randomized controlled trial of highly active antiretroviral therapy versus highly active antiretroviral therapy and chemotherapy in therapy-naive patients with HIV-associated Kaposi sarcoma in South Africa. J Acquir Immune Defic Syndr 60:150–157

Moscicki AB, Ma Y, Holland C, Vermund SH (2001) Cervical ectopy in adolescent girls with and without human immunodeficiency virus infection. J Infect Dis 183:865–870

Moss GB, Clemetson D, D'costa L, Plummer FA, Ndinya-Achola JO, Reilly M, Holmes KK, Piot P, Maitha GM, Hillier SL et al (1991) Association of cervical ectopy with heterosexual transmission of human immunodeficiency virus: results of a study of couples in Nairobi, Kenya. J Infect Dis 164:588–591

Mostad SB, Overbaugh J, Devange DM, Welch MJ, Chohan B, Mandaliya K, Nyange P, Martin HL Jr, Ndinya-Achola J, Bwayo JJ, Kreiss JK (1997) Hormonal contraception, vitamin A deficiency, and other risk factors for shedding of HIV-1 infected cells from the cervix and vagina. Lancet 350:922–927

Mugo NR, Heffron R, Donnell D, Wald A, Were EO, Rees H, Celum C, Kiarie JN, Cohen CR, Kayintekore K, Baeten JM (2011) Increased risk of HIV-1 transmission in pregnancy: a prospective study among African HIV-1-serodiscordant couples. AIDS 25:1887–1895

Munch J, Rucker E, Standker L, Adermann K, Goffinet C, Schindler M, Wildum S, Chinnadurai R, Rajan D, Specht A, Gimenez-Gallego G, Sanchez PC, Fowler DM, Koulov A, Kelly JW, Mothes W, Grivel JC, Margolis L, Keppler OT, Forssmann WG, Kirchhoff F (2007) Semen-derived amyloid fibrils drastically enhance HIV infection. Cell 131:1059–1071

Muntemba S, Githagui N, Chesoni A, Muragori BW, Maina B, Kibiti R (2003) The Kenya Strategic Country Gender Assessment. Prem and Essd- Africa Region, World Bank

Mureith MW, Chang JJ, Lifson JD, Ndung'U T, Altfeld M (2010) Exposure to HIV-1-encoded Toll-like receptor 8 ligands enhances monocyte response to microbial encoded Toll-like receptor 2/4 ligands. AIDS 24:1841–1848

Murri R, Lepri AC, Phillips AN, Girardi E, Nasti G, Ferrara S, Mura MS, Mussini C, Petrelli E, Arlotti M, De Stefano C, Vigano P, Novati R, Cargnel A, Monforte AD (2003) Access to antiretroviral treatment, incidence of sustained therapy interruptions, and risk of clinical events according to sex: evidence from the. J Acquir Immune Defic Syndr 34:184–190

Myer L, Denny L, Wright TC, Kuhn L (2007) Prospective study of hormonal contraception and women's risk of HIV infection in South Africa. Int J Epidemiol 36:166–174

Myer L, Wright TC Jr, Denny L, Kuhn L (2006) Nested case-control study of cervical mucosal lesions, ectopy, and incident HIV infection among women in Cape Town, South Africa. Sex Transm Dis 33:683–687

Nag P, Kim J, Sapiega V, Landay AL, Bremer JW, Mestecky J, Reichelderfer P, Kovacs A, Cohn J, Weiser B, Baum LL (2004) Women with cervicovaginal antibody-dependent cell-mediated cytotoxicity have lower genital HIV-1 RNA loads. J Infect Dis 190:1970–1978

Napravnik S, Poole C, Thomas JC, Eron JJ Jr (2002) Gender difference in HIV RNA levels: a meta-analysis of published studies. J Acquir Immune Defic Syndr 31:11–19

Naranbhai V, Abdool Karim SS, Altfeld M, Samsunder N, Durgiah R, Sibeko S, Abdool Karim Q, Carr WH (2012) Innate immune activation enhances HIV acquisition in women, diminishing the effectiveness of tenofovir microbicide gel. J Infect Dis 206:993–1001

Nardelli-Haefliger D, Wirthner D, Schiller JT, Lowy DR, Hildesheim A, Ponci F, De Grandi P (2003) Specific antibody levels at the cervix during the menstrual cycle of women vaccinated with human papillomavirus 16 virus-like particles. J Natl Cancer Inst 95:1128–1137

Nash D, Katyal M, Brinkhof MW, Keiser O, May M, Hughes R, Dabis F, Wood R, Sprinz E, Schechter M, Egger M (2008) Long-term immunologic response to antiretroviral therapy in low-income countries: a collaborative analysis of prospective studies. AIDS 22:2291–2302

Nasti G, Serraino D, Ridolfo A, Antinori A, Rizzardini G, Zeroli C, Nigro L, Tavio M, Vaccher E, Tirelli U (1999) Aids-associated Kaposi's sarcoma is more aggressive in women: a study of 54 patients. J Acquir Immune Defic Syndr Hum Retrovirol 20:337–341

Nglazi MD, Lawn SD, Kaplan R, Kranzer K, Orrell C, Wood R, Bekker LG (2011) Changes in programmatic outcomes during 7 years of scale-up at a community-based antiretroviral treatment service in South Africa. J Acquir Immune Defic Syndr 56:e1–e8

Nicastri E, Angeletti C, Palmisano L, Sarmati L, Chiesi A, Geraci A, Andreoni M, Vella S (2005) Gender differences in clinical progression of HIV-1-infected individuals during long-term highly active antiretroviral therapy. AIDS 19:577–583

Niccolai LM, Kopicko JJ, Kassie A, Petros H, Clark RA, Kissinger P (2000) Incidence and predictors of reinfection with Trichomonas vaginalis in HIV-infected women. Sex Transm Dis 27:284–288

Nicolosi A, Correa Leite ML, Musicco M, Arici C, Gavazzeni G, Lazzarin A (1994) The efficiency of male-to-female and female-to-male sexual transmission of the human immunodeficiency virus: a study of 730 stable couples. Italian Study Group on HIV Heterosexual Transmission. Epidemiology 5:570–575

Nilsson K, Heimer G (1992) Endogenous estrogen levels in postmenopausal women with severe urogenital atrophy. Gynecol Obstet Invest 34:234–236

Nissen SK, Hojen JF, Andersen KL, Kofod-Olsen E, Berg RK, Paludan SR, Ostergaard L, Jakobsen MR, Tolstrup M, Mogensen TH (2014) Innate Dna sensing is impaired in HIV patients and Ifi16 expression correlates to chronic immune activation. Clin Exp Immunol 177:295–307

Njai HF, Ewings FM, Lyimo E, Foulongne V, Ngerageza D, Mongi A, Ssemwanga D, Andreasen A, Nyombi B, Ao T, Michael D, Urassa M, Todd J, Zaba B, Changalucha J, Hayes R, Kapiga SH (2013) Deciphering the Complex Distribution of Human Immunodeficiency Virus Type 1 Subtypes among Different Cohorts in Northern Tanzania. PLoS One 8: e81848

Nordone SK, Ignacio GA, Su L, Sempowski GD, Golenbock DT, Li L, Dean GA (2007) Failure of Tlr4-driven Nf-kappa B activation to stimulate virus replication in models of HIV type 1 activation. AIDS Res Hum Retroviruses 23:1387–1395

Norman R, Matzopoulos R, Groenewald P, Bradshaw D (2007) The high burden of injuries in South Africa. Bull World Health Organ 85:695–702

Norrgren H, Da Silva ZJ, Andersson S, Biague AJ, Dias F, Biberfeld G, Naucler A (1998) Clinical features, immunological changes and mortality in a cohort of HIV-2-infected individuals in Bissau, Guinea-Bissau. Scand J Infect Dis 30:323–329

Nyamweya S, Hegedus A, Jaye A, Rowland-Jones S, Flanagan KL, Macallan DC (2013) Comparing HIV-1 and HIV-2 infection: Lessons for viral immunopathogenesis. Rev Med Virol 23: 221–240

O'brien M, Manches O, Bhardwaj N (2013) Plasmacytoid dendritic cells in HIV infection. Adv Exp Med Biol 762:71–107

O'brien M, Manches O, Sabado RL, Baranda SJ, Wang Y, Marie I, Rolnitzky L, Markowitz M, Margolis DM, Levy D, Bhardwaj N (2011) Spatiotemporal trafficking of HIV in human plasmacytoid dendritic cells defines a persistently Ifn-alpha-producing and partially matured phenotype. J Clin Invest 121:1088–1101

O'brien TR, Busch MP, Donegan E, Ward JW, Wong L, Samson SM, Perkins HA, Altman R, Stoneburner RL, Holmberg SD (1994) Heterosexual transmission of human immunodeficiency

virus type 1 from transfusion recipients to their sex partners. J Acquir Immune Defic Syndr 7: 705–710
O'farrell N (2001) Enhanced efficiency of female-to-male HIV transmission in core groups in developing countries: the need to target men. Sex Transm Dis 28:84–91
Pa T (1988) Aids, gender and biomedical discourse: current contests for meaning. In: Fee E, Fox DM (eds) Aids: Burdens of History. University of California Press, Berkeley, CA
Paavonen T (1994) Hormonal regulation of immune responses. Ann Med 26:255–258
Padian NS, Shiboski SC, Jewell NP (1990) The effect of number of exposures on the risk of heterosexual HIV transmission. J Infect Dis 161:883–887
Padian NS, Shiboski SC, Jewell NP (1991) Female-to-male transmission of human immunodeficiency virus. JAMA 266:1664–1667
Palefsky J (2009) Human papillomavirus-related disease in people with HIV. Curr Opin HIV AIDS 4:52–56
Palella FJ Jr, Baker RK, Moorman AC, Chmiel JS, Wood KC, Brooks JT, Holmberg SD (2006) Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr 43:27–34
Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD (1998) Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 338: 853–860
Panchanathan R, Shen H, Bupp MG, Gould KA, Choubey D (2009) Female and male sex hormones differentially regulate expression of Ifi202, an interferon-inducible lupus susceptibility gene within the NBA2 interval. J Immunol 183:7031–7038
Pancino G, Saez-Cirion A, Scott-Algara D, Paul P (2010) Natural resistance to HIV infection: lessons learned from HIV-exposed uninfected individuals. J Infect Dis 202(Suppl 3): S345–S350
Paramsothy P, Jamieson DJ, Heilig CM, Schuman PC, Klein RS, Shah KV, Rompalo AM, Cu-Uvin S, Duerr A (2009) The effect of highly active antiretroviral therapy on human papillomavirus clearance and cervical cytology. Obstet Gynecol 113:26–31
Patterson K, Napravnik S, Eron J, Keruly J, Moore R (2007) Effects of age and sex on immunological and virological responses to initial highly active antiretroviral therapy. HIV Med 8: 406–410
Patterson KB, Cohn SE, Uyanik J, Hughes M, Smurzynski M, Eron JJ (2009) Treatment responses in antiretroviral treatment-naive premenopausal and postmenopausal HIV-1-infected women: an analysis from Aids Clinical Trials Group Studies. Clin Infect Dis 49:473–476
Patton DL, Thwin SS, Meier A, Hooton TM, Stapleton AE, Eschenbach DA (2000) Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle. Am J Obstet Gynecol 183:967–973
Perez-Hoyos S, Del Amo J, Muga R, Del Romero J, Garcia De Olalla P, Guerrero R, Hernandez-Aguado I (2003) Effectiveness of highly active antiretroviral therapy in Spanish cohorts of HIV seroconverters: differences by transmission category. AIDS 17:353–359
Peterman TA, Curran JW (1986) Sexual transmission of human immunodeficiency virus. JAMA 256:2222–2226
Peterman TA, Stoneburner RL, Allen JR, Jaffe HW, Curran JW (1988) Risk of human immunodeficiency virus transmission from heterosexual adults with transfusion-associated infections. JAMA 259:55–58
Phillips AN, Staszewski S, Weber R, Kirk O, Francioli P, Miller V, Vernazza P, Lundgren JD, Ledergerber B (2001) HIV viral load response to antiretroviral therapy according to the baseline Cd4 cell count and viral load. JAMA 286:2560–2567
Phillips SJ, Curtis KM, Polis CB (2013) Effect of hormonal contraceptive methods on HIV disease progression: a systematic review. AIDS 27:787–794

Pilcher CD, Tien HC, Eron JJ Jr, Vernazza PL, Leu SY, Stewart PW, Goh LE, Cohen MS (2004) Brief but efficient: acute HIV infection and the sexual transmission of HIV. J Infect Dis 189: 1785–1792

Pillay T, Khan M, Moodley J, Adhikari M, Coovadia H (2004) Perinatal tuberculosis and HIV-1: considerations for resource-limited settings. Lancet Infect Dis 4:155–165

Piot P, Laga M (1989) Genital ulcers, other sexually transmitted diseases, and the sexual transmission of HIV. BMJ 298:623–624

Piot P, Quinn TC, Taelman H, Feinsod FM, Minlangu KB, Wobin O, Mbendi N, Mazebo P, Ndangi K, Stevens W et al (1984) Acquired immunodeficiency syndrome in a heterosexual population in Zaire. Lancet 2:65–69

Piroth L, Grappin M, Cuzin L, Mouton Y, Bouchard O, Raffi F, Rey D, Peyramond D, Gourdon F, Drobacheff C, Lombart ML, Lucht F, Besnier JM, Bernard L, Chavanet P, Portier H (2000) Hepatitis C virus co-infection is a negative prognostic factor for clinical evolution in human immunodeficiency virus-positive patients. J Viral Hepat 7:302–308

Piwoz EG, Humphrey JH, Marinda ET, Mutasa K, Moulton LH, Iliff PJ (2006) Effects of infant sex on mother-to-child transmission of HIV-1 according to timing of infection in Zimbabwe. AIDS 20:1981–1984

Plourde PJ, Pepin J, Agoki E, Ronald AR, Ombette J, Tyndall M, Cheang M, Ndinya-Achola JO, D'costa LJ, Plummer FA (1994) Human immunodeficiency virus type 1 seroconversion in women with genital ulcers. J Infect Dis 170:313–317

Plourde PJ, Plummer FA, Pepin J, Agoki E, Moss G, Ombette J, Ronald AR, Cheang M, D'costa L, Ndinya-Achola JO (1992) Human immunodeficiency virus type 1 infection in women attending a sexually transmitted diseases clinic in Kenya. J Infect Dis 166:86–92

Plummer FA, Simonsen JN, Cameron DW, Ndinya-Achola JO, Kreiss JK, Gakinya MN, Waiyaki P, Cheang M, Piot P, Ronald AR et al (1991) Cofactors in male-female sexual transmission of human immunodeficiency virus type 1. J Infect Dis 163:233–239

Poonia B, Walter L, Dufour J, Harrison R, Marx PA, Veazey RS (2006) Cyclic changes in the vaginal epithelium of normal rhesus macaques. J Endocrinol 190:829–835

Porco TC, Martin JN, Page-Shafer KA, Cheng A, Charlebois E, Grant RM, Osmond DH (2004) Decline in HIV infectivity following the introduction of highly active antiretroviral therapy. AIDS 18:81–88

Porter K, Babiker A, Bhaskaran K, Darbyshire J, Pezzotti P, Porter K, Walker AS (2003) Determinants of survival following HIV-1 seroconversion after the introduction of Haart. Lancet 362:1267–1274

Poss M, Martin HL, Kreiss JK, Granville L, Chohan B, Nyange P, Mandaliya K, Overbaugh J (1995) Diversity in virus populations from genital secretions and peripheral blood from women recently infected with human immunodeficiency virus type 1. J Virol 69:8118–8122

Poulsen AG, Aaby P, Larsen O, Jensen H, Naucler A, Lisse IM, Christiansen CB, Dias F, Melbye M (1997) 9-year HIV-2-associated mortality in an urban community in Bissau, west Africa. Lancet 349:911–914

Poundstone KE, Chaisson RE, Moore RD (2001) Differences in HIV disease progression by injection drug use and by sex in the era of highly active antiretroviral therapy. AIDS 15:1115–1123

Powers KA, Poole C, Pettifor AE, Cohen MS (2008) Rethinking the heterosexual infectivity of HIV-1: a systematic review and meta-analysis. Lancet Infect Dis 8:553–563

Poynard T, Bedossa P, Opolon P (1997) Natural history of liver fibrosis progression in patients with chronic hepatitis C. The Obsvirc, Metavir, Clinivir, and Dosvirc groups. Lancet 349: 825–832

Prakash M, Kapembwa MS, Gotch F, Patterson S (2002) Oral contraceptive use induces upregulation of the Ccr5 chemokine receptor on Cd4(+) T cells in the cervical epithelium of healthy women. J Reprod Immunol 54:117–131

Prins M, Meyer L, Hessol NA (2005) Sex and the course of HIV infection in the pre- and highly active antiretroviral therapy eras. AIDS 19:357–370

Prins M, Robertson JR, Brettle RP, Aguado IH, Broers B, Boufassa F, Goldberg DJ, Zangerle R, Coutinho RA, Van Den Hoek A (1999) Do gender differences in Cd4 cell counts matter? AIDS 13:2361–2364

Prodger JL, Gray R, Kigozi G, Nalugoda F, Galiwango R, Hirbod T, Wawer M, Hofer SO, Sewankambo N, Serwadda D, Kaul R (2012) Foreskin T-cell subsets differ substantially from blood with respect to HIV co-receptor expression, inflammatory profile, and memory status. Mucosal Immunol 5:121–128

Pudney J, Quayle AJ, Anderson DJ (2005) Immunological microenvironments in the human vagina and cervix: mediators of cellular immunity are concentrated in the cervical transformation zone. Biol Reprod 73:1253–1263

Quinn TC (1994) Population migration and the spread of types 1 and 2 human immunodeficiency viruses. Proc Natl Acad Sci U S A 91:2407–2414

Quinn TC, Mann JM, Curran JW, Piot P (1986) Aids in Africa: an epidemiologic paradigm. Science 234:955–963

Quinn TC, Overbaugh J (2005) HIV/Aids in women: an expanding epidemic. Science 308: 1582–1583

Quinn TC, Wawer MJ, Sewankambo N, Serwadda D, Li C, Wabwire-Mangen F, Meehan MO, Lutalo T, Gray RH (2000) Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. N Engl J Med 342:921–929

Rabkin JG, Johnson J, Lin SH, Lipsitz JD, Remien RH, Williams JB, Gorman JM (1997) Psychopathology in male and female HIV-positive and negative injecting drug users: longitudinal course over 3 years. AIDS 11:507–515

Raboud J, Blitz S, Walmsley S, Thompson C, Rourke SB, Loutfy MR (2010) Effect of gender and calendar year on time to and duration of virologic suppression among antiretroviral-naive HIV-infected individuals initiating combination antiretroviral therapy. HIV Clin Trials 11: 340–350

Rachlis AR, Zarowny DP (1998) Guidelines for antiretroviral therapy for HIV infection. Canadian HIV Trials Network Antiretroviral Working Group. CMAJ 158:496–505

Radzio J, Aung W, Holder A, Martin A, Sweeney E, Mitchell J, Bachman S, Pau CP, Heneine W, Garcia-Lerma JG (2012) Prevention of vaginal SHIV transmission in macaques by a coitally-dependent Truvada regimen. PLoS One 7:e50632

Radzio J, Hanley K, Mitchell J, Ellis S, Deyounks F, Jenkins LT, Hanson D, Heneine W, Garcia-Lerma JG (2014) Physiologic doses of depot-medroxyprogesterone acetate do not increase acute plasma simian HIV viremia or mucosal virus shedding in pigtail macaques. AIDS 28: 1431–1439

Ragupathy V, Devadas K, Tang S, Wood O, Lee S, Dastyer A, Wang X, Dayton A, Hewlett I (2013) Effect of sex steroid hormones on replication and transmission of major HIV subtypes. J Steroid Biochem Mol Biol 138:63–71

Ratner Kaufman F, Gertner JM, Sleeper LA, Donfield SM (1997) Growth hormone secretion in HIV-positive versus HIV-negative hemophilic males with abnormal growth and pubertal development. The Hemophilia Growth and Development Study. J Acquir Immune Defic Syndr Hum Retrovirol 15:137–144

Ray K, Gupta SM, Bala M, Muralidhar S, Kumar J (2006) Cd4/Cd8 lymphocyte counts in healthy, HIV-positive individuals & Aids patients. Indian J Med Res 124:319–330

Rebbapragada A, Wachihi C, Pettengell C, Sunderji S, Huibner S, Jaoko W, Ball B, Fowke K, Mazzulli T, Plummer FA, Kaul R (2007) Negative mucosal synergy between Herpes simplex type 2 and HIV in the female genital tract. AIDS 21:589–598

Redfield RR, Markham PD, Salahuddin SZ, Wright DC, Sarngadharan MG, Gallo RC (1985) Heterosexually acquired HTLV-III/LAV disease (Aids-related complex and Aids). Epidemiologic evidence for female-to-male transmission. JAMA 254:2094–2096

Rehle T, Brinkmann UK, Siraprapasiri T, Coplan P, Aiemsukawat C, Ungchusak K (1992) Risk factors of HIV-1 infection among female prostitutes in Khon Kaen, Northeast Thailand. Infection 20:328–331

Reichelderfer PS, Coombs RW, Wright DJ, Cohn J, Burns DN, Cu-Uvin S, Baron PA, Coheng MH, Landay AL, Beckner SK, Lewis SR, Kovacs AA (2000) Effect of menstrual cycle on HIV-1 levels in the peripheral blood and genital tract. Whs 001 Study Team. AIDS 14:2101–2107

Reichert T, Debruyere M, Deneys V, Totterman T, Lydyard P, Yuksel F, Chapel H, Jewell D, Van Hove L, Linden J et al (1991) Lymphocyte subset reference ranges in adult Caucasians. Clin Immunol Immunopathol 60:190–208

Reid SE, Dai JY, Wang J, Sichalwe BN, Akpomiemie G, Cowan FM, Delany-Moretlwe S, Baeten JM, Hughes JP, Wald A, Celum C (2010) Pregnancy, contraceptive use, and HIV acquisition in Hptn 039: relevance for HIV prevention trials among African women. J Acquir Immune Defic Syndr 53:606–613

Reynolds SJ, Quinn TC (2005) Developments in Std/HIV interactions: the intertwining epidemics of HIV and Hsv-2. Infect Dis Clin North Am 19:415–425

Reynolds SJ, Risbud AR, Shepherd ME, Zenilman JM, Brookmeyer RS, Paranjape RS, Divekar AD, Gangakhedkar RR, Ghate MV, Bollinger RC, Mehendale SM (2003) Recent herpes simplex virus type 2 infection and the risk of human immunodeficiency virus type 1 acquisition in India. J Infect Dis 187:1513–1521

Ribera E, Lopez RM, Diaz M, Pou L, Ruiz L, Falco V, Crespo M, Azuaje C, Ruiz I, Ocana I, Clotet B, Pahissa A (2004) Steady-state pharmacokinetics of a double-boosting regimen of saquinavir soft gel plus lopinavir plus minidose ritonavir in human immunodeficiency virus-infected adults. Antimicrob Agents Chemother 48:4256–4262

Ricard D, Wilkins A, N'gum PT, Hayes R, Morgan G, Da Silva AP, Whittle H (1994) The effects of HIV-2 infection in a rural area of Guinea-Bissau. AIDS 8:977–982

Richardson BA, Mbori-Ngacha D, Lavreys L, John-Stewart GC, Nduati R, Panteleeff DD, Emery S, Kreiss JK, Overbaugh J (2003) Comparison of human immunodeficiency virus type 1 viral loads in Kenyan women, men, and infants during primary and early infection. J Virol 77:7120–7123

Richardson BA, Morrison CS, Sekadde-Kigondu C, Sinei SK, Overbaugh J, Panteleeff DD, Weiner DH, Kreiss JK (1999) Effect of intrauterine device use on cervical shedding of HIV-1 DNA. AIDS 13:2091–2097

Richardson BA, Otieno PA, Mbori-Ngacha D, Overbaugh J, Farquhar C, John-Stewart GC (2007) Hormonal contraception and HIV-1 disease progression among postpartum Kenyan women. AIDS 21:749–753

Ritola K, Pilcher CD, Fiscus SA, Hoffman NG, Nelson JA, Kitrinos KM, Hicks CB, Eron JJ Jr, Swanstrom R (2004) Multiple V1/V2 env variants are frequently present during primary infection with human immunodeficiency virus type 1. J Virol 78:11208–11218

Roberts L, Passmore JA, Mlisana K, Williamson C, Little F, Bebell LM, Walzl G, Abrahams MR, Woodman Z, Abdool Karim Q, Abdool Karim SS (2012) Genital tract inflammation during early HIV-1 infection predicts higher plasma viral load set point in women. J Infect Dis 205:194–203

Rodriguez-Torres M, Rios-Bedoya CF, Rodriguez-Orengo J, Fernandez-Carbia A, Marxuach-Cuetara AM, Lopez-Torres A, Salgado-Mercado R, Brau N (2006) Progression to cirrhosis in Latinos with chronic hepatitis C: differences in Puerto Ricans with and without human immunodeficiency virus coinfection and along gender. J Clin Gastroenterol 40:358–366

Rotchford K, Strum AW, Wilkinson D (2000) Effect of coinfection with Stds and of Std treatment on HIV shedding in genital-tract secretions: systematic review and data synthesis. Sex Transm Dis 27:243–248

Rothenberg R, Woelfel M, Stoneburner R, Milberg J, Parker R, Truman B (1987) Survival with the acquired immunodeficiency syndrome. Experience with 5833 cases in New York City. N Engl J Med 317:1297–1302

Rottingen JA, Cameron DW, Garnett GP (2001) A systematic review of the epidemiologic interactions between classic sexually transmitted diseases and HIV: how much really is known? Sex Transm Dis 28:579–597

Rowland-Jones SL, Whittle HC (2007) Out of Africa: what can we learn from HIV-2 about protective immunity to HIV-1? Nat Immunol 8:329–331

Saada M, Le Chenadec J, Berrebi A, Bongain A, Delfraissy JF, Mayaux MJ, Meyer L (2000) Pregnancy and progression to Aids: results of the French prospective cohorts. Serogest and Seroco Study Groups. AIDS 14:2355–2360

Sabine C (2005) Aids events among individuals initiating Haart: do some patients experience a greater benefit from Haart than others? AIDS 19:1995–2000

Sackoff JE, Hanna DB, Pfeiffer MR, Torian LV (2006) Causes of death among persons with Aids in the era of highly active antiretroviral therapy: New York City. Ann Intern Med 145:397–406

Sagar M, Kirkegaard E, Long EM, Celum C, Buchbinder S, Daar ES, Overbaugh J (2004a) Human immunodeficiency virus type 1 (HIV-1) diversity at time of infection is not restricted to certain risk groups or specific HIV-1 subtypes. J Virol 78:7279–7283

Sagar M, Lavreys L, Baeten JM, Richardson BA, Mandaliya K, Ndinya-Achola JO, Kreiss JK, Overbaugh J (2004b) Identification of modifiable factors that affect the genetic diversity of the transmitted HIV-1 population. AIDS 18:615–619

Salazar-Gonzalez JF, Salazar MG, Keele BF, Learn GH, Giorgi EE, Li H, Decker JM, Wang S, Baalwa J, Kraus MH, Parrish NF, Shaw KS, Guffey MB, Bar KJ, Davis KL, Ochsenbauer-Jambor C, Kappes JC, Saag MS, Cohen MS, Mulenga J, Derdeyn CA, Allen S, Hunter E, Markowitz M, Hraber P, Perelson AS, Bhattacharya T, Haynes BF, Korber BT, Hahn BH, Shaw GM (2009) Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. J Exp Med 206:1273–1289

Salem ML, Hossain MS, Nomoto K (2000) Mediation of the immunomodulatory effect of beta-estradiol on inflammatory responses by inhibition of recruitment and activation of inflammatory cells and their gene expression of TNF-alpha and IFN-gamma. Int Arch Allergy Immunol 121:235–245

Sandelowski M, Barroso J, Voils CI (2009) Gender, race/ethnicity, and social class in research reports on stigma in HIV-positive women. Health Care Women Int 30:273–288

Sankaran-Walters S, Macal M, Grishina I, Nagy L, Goulart L, Coolidge K, Li J, Fenton A, Williams T, Miller MK, Flamm J, Prindiville T, George M, Dandekar S (2013) Sex differences matter in the gut: effect on mucosal immune activation and inflammation. Biol Sex Differ 4:10

Saves M, Morlat P, Chene G, Dumon B, Peuchant E (1999) Sex differences in HIV-1 viral load and TNF-alpha plasmatic level? AIDS 13:1414–1415

Schoenbaum EE, Hartel D, Lo Y, Howard AA, Floris-Moore M, Arnsten JH, Santoro N (2005) HIV infection, drug use, and onset of natural menopause. Clin Infect Dis 41:1517–1524

Seidlin M, Vogler M, Lee E, Lee YS, Dubin N (1993) Heterosexual transmission of HIV in a cohort of couples in New York City. AIDS 7:1247–1254

Seillet C, Laffont S, Tremollieres F, Rouquie N, Ribot C, Arnal JF, Douin-Echinard V, Gourdy P, Guery JC (2012) The Tlr-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor alpha signaling. Blood 119:454–464

Selwyn PA, Schoenbaum EE, Davenny K, Robertson VJ, Feingold AR, Shulman JF, Mayers MM, Klein RS, Friedland GH, Rogers MF (1989) Prospective study of human immunodeficiency virus infection and pregnancy outcomes in intravenous drug users. JAMA 261:1289–1294

Semaille C, Barin F, Cazein F, Pillonel J, Lot F, Brand D, Plantier JC, Bernillon P, Le Vu S, Pinget R, Desenclos JC (2007) Monitoring the dynamics of the HIV epidemic using assays for recent infection and serotyping among new HIV diagnoses: experience after 2 years in France. J Infect Dis 196:377–383

Semple SJ, Patterson TL, Straits-Troster K, Atkinson JH, Mccutchan JA, Grant I (1996) Social and psychological characteristics of HIV-infected women and gay men. HIV Neurobehavioral Research Center (HNRC) Group. Women Health 24:17–41

Serrano-Villar S, Gutierrez C, Vallejo A, Hernandez-Novoa B, Diaz L, Abad Fernandez M, Madrid N, Dronda F, Zamora J, Munoz-Fernandez MA, Moreno S (2013) The Cd4/Cd8 ratio

in HIV-infected subjects is independently associated with T-cell activation despite long-term viral suppression. J Infect 66:57–66
Sexton J, Garnett G, Rottingen JA (2005) Metaanalysis and metaregression in interpreting study variability in the impact of sexually transmitted diseases on susceptibility to HIV infection. Sex Transm Dis 32:351–357
Shanker G, Sorci-Thomas M, Adams MR (1994) Estrogen modulates the expression of tumor necrosis factor alpha mRNA in phorbol ester-stimulated human monocytic Thp-1 cells. Lymphokine Cytokine Res 13:377–382
Shapira-Nahor O, Kalinkovich A, Weisman Z, Greenberg Z, Nahmias J, Shapiro M, Panet A, Bentwich Z (1998) Increased susceptibility to HIV-1 infection of peripheral blood mononuclear cells from chronically immune-activated individuals. AIDS 12:1731–1733
Sheffield JS, Wendel GD Jr, Mcintire DD, Norgard MV (2009) The effect of progesterone levels and pregnancy on HIV-1 coreceptor expression. Reprod Sci 16:20–31
Shepherd ME, Gangakhedkar RR, Sahay S, Reynolds SJ, Ghate MV, Risbud AR, Paranjape RS, Bollinger RC, Mehendale SM (2003) Incident HIV infection among men attending Std clinics in Pune, India: pathways to disparity and interventions to enhance equity. J Health Popul Nutr 21:251–263
Sherman KE, Rouster SD, Chung RT, Rajicic N (2002) Hepatitis C Virus prevalence among patients infected with Human Immunodeficiency Virus: a cross-sectional analysis of the US adult Aids Clinical Trials Group. Clin Infect Dis 34:831–837
Sheth PM, Kovacs C, Kemal KS, Jones RB, Raboud JM, Pilon R, La Porte C, Ostrowski M, Loutfy M, Burger H, Weiser B, Kaul R (2009) Persistent HIV RNA shedding in semen despite effective antiretroviral therapy. AIDS 23:2050–2054
Sheth PM, Yi TJ, Kovacs C, Kemal KS, Jones RB, Osborne B, Pilon R, La Porte C, Ostrowski M, Mazzulli T, Burger H, Weiser B, Kaul R (2012) Mucosal correlates of isolated HIV semen shedding during effective antiretroviral therapy. Mucosal Immunol 5:248–257
Shiboski S, Padian NS (1996) Population- and individual-based approaches to the design and analysis of epidemiologic studies of sexually transmitted disease transmission. J Infect Dis 174 (Suppl 2):S188–S200
Shiels MS, Cole SR, Kirk GD, Poole C (2009) A meta-analysis of the incidence of non-Aids cancers in HIV-infected individuals. J Acquir Immune Defic Syndr 52:611–622
Shiels MS, Pfeiffer RM, Engels EA (2010) Age at cancer diagnosis among persons with Aids in the United States. Ann Intern Med 153:452–460
Shrier LA, Bowman FP, Lin M, Crowley-Nowick PA (2003) Mucosal immunity of the adolescent female genital tract. J Adolesc Health 32:183–186
Siddiqui RA, Sauermann U, Altmuller J, Fritzer E, Nothnagel M, Dalibor N, Fellay J, Kaup FJ, Stahl-Hennig C, Nurnberg P, Krawczak M, Platzer M (2009) X chromosomal variation is associated with slow progression to Aids in HIV-1-infected women. Am J Hum Genet 85: 228–239
Sinei SK, Fortney JA, Kigondu CS, Feldblum PJ, Kuyoh M, Allen MY, Glover LH (1996) Contraceptive use and HIV infection in Kenyan family planning clinic attenders. Int J STD AIDS 7:65–70
Slaughter L, Brown CR, Crowley S, Peck R (1997) Patterns of genital injury in female sexual assault victims. Am J Obstet Gynecol 176:609–616
Small PM (2009) Tuberculosis: a new vision for the 21st century. Kekkaku 84:721–726
Smit C, Geskus R, Walker S, Sabin C, Coutinho R, Porter K, Prins M (2006) Effective therapy has altered the spectrum of cause-specific mortality following HIV seroconversion. AIDS 20: 741–749
Smith J, Nalagoda F, Wawer MJ, Serwadda D, Sewankambo N, Konde-Lule J, Lutalo T, Li C, Gray RH (1999) Education attainment as a predictor of HIV risk in rural Uganda: results from a population-based study. Int J STD AIDS 10:452–459
Smith MM, Kuhn L (2000) Exclusive breast-feeding: does it have the potential to reduce breast-feeding transmission of HIV-1? Nutr Rev 58:333–340

Smith P (1993) Estrogens and the urogenital tract. Studies on steroid hormone receptors and a clinical study on a new estradiol-releasing vaginal ring. Acta Obstet Gynecol Scand Suppl 157: 1–26

Smith SM, Baskin GB, Marx PA (2000) Estrogen protects against vaginal transmission of simian immunodeficiency virus. J Infect Dis 182:708–715

Smith SM, Mefford M, Sodora D, Klase Z, Singh M, Alexander N, Hess D, Marx PA (2004) Topical estrogen protects against SIV vaginal transmission without evidence of systemic effect. AIDS 18:1637–1643

Smith-Franklin BA, Keele BF, Tew JG, Gartner S, Szakal AK, Estes JD, Thacker TC, Burton GF (2002) Follicular dendritic cells and the persistence of HIV infectivity: the role of antibodies and Fcgamma receptors. J Immunol 168:2408–2414

Sodora DL, Gettie A, Miller CJ, Marx PA (1998) Vaginal transmission of SIV: assessing infectivity and hormonal influences in macaques inoculated with cell-free and cell-associated viral stocks. AIDS Res Hum Retroviruses 14(Suppl 1):S119–S123

Somdyala NI, Bradshaw D, Gelderblom WC, Parkin DM (2010) Cancer incidence in a rural population of South Africa, 1998-2002. Int J Cancer 127:2420–2429

Soon GG, Min M, Struble KA, Chan-Tack KM, Hammerstrom T, Qi K, Zhou S, Bhore R, Murray JS, Birnkrant DB (2012) Meta-analysis of gender differences in efficacy outcomes for HIV-positive subjects in randomized controlled clinical trials of antiretroviral therapy (2000–2008). AIDS Patient Care STDS 26:444–453

Sonnex C (1998) Influence of ovarian hormones on urogenital infection. Sex Transm Infect 74: 11–19

Sorensen K, Mouritsen A, Aksglaede L, Hagen CP, Mogensen SS, Juul A (2012) Recent secular trends in pubertal timing: implications for evaluation and diagnosis of precocious puberty. Horm Res Paediatr 77:137–145

Soto-Ramirez LE, Renjifo B, Mclane MF, Marlink R, O'Hara C, Sutthent R, Wasi C, Vithayasai P, Vithayasai V, Apichartpiyakul C, Auewarakul P, Pena Cruz V, Chui DS, Osathanondh R, Mayer K, Lee TH, Essex M (1996) HIV-1 Langerhans' cell tropism associated with heterosexual transmission of HIV. Science 271:1291–1293

Soto B, Sanchez-Quijano A, Rodrigo L, Del Olmo JA, Garcia-Bengoechea M, Hernandez-Quero J, Rey C, Abad MA, Rodriguez M, Sales Gilabert M, Gonzalez F, Miron P, Caruz A, Relimpio F, Torronteras R, Leal M, Lissen E (1997) Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. J Hepatol 26:1–5

Spierings E, Vermeulen CJ, Vogt MH, Doerner LE, Falkenburg JH, Mutis T, Goulmy E (2003) Identification of Hla class Ii-restricted H-Y-specific T-helper epitope evoking Cd4+ T-helper cells in H-Y-mismatched transplantation. Lancet 362:610–615

Stagi S, Galli L, Cecchi C, Chiappini E, Losi S, Gattinara CG, Gabiano C, Tovo PA, Bernardi S, Chiarelli F, De Martino M (2010) Final height in patients perinatally infected with the human immunodeficiency virus. Horm Res Paediatr 74:165–171

Stanley M (2009) Early age of sexual debut: a risky experience. J Fam Plann Reprod Health Care 35:118–120

Staples CT, Rimland D, Dudas D (1999) Hepatitis C in the HIV (human immunodeficiency virus) Atlanta V.A. (Veterans Affairs Medical Center) Cohort Study (Havacs): the effect of coinfection on survival. Clin Infect Dis 29:150–154

Stark K, Poggensee G, Hohne M, Bienzle U, Kiwelu I, Schreier E (2000) Seroepidemiology of Tt virus, Gbc-C/Hgv, and hepatitis viruses B, C, and E among women in a rural area of Tanzania. J Med Virol 62:524–530

Steiner RA, Schiller HS, Illner P, Blandau R, Gale CC (1977) Sex hormones correlated with sex skin swelling and rectal temperature during the menstrual cycle of the pigtail macaque (Macaca nemestrina). Lab Anim Sci 27:217–221

Stenehjem E, Shlay JC (2008) Sex-specific differences in treatment outcomes for patients with HIV and AIDS. Expert Rev Pharmacoecon Outcomes Res 8:51–63

Sterling TR, Lyles CM, Vlahov D, Astemborski J, Margolick JB, Quinn TC (1999) Sex differences in longitudinal human immunodeficiency virus type 1 Rna levels among seroconverters. J Infect Dis 180:666–672

Sterling TR, Vlahov D, Astemborski J, Hoover DR, Margolick JB, Quinn TC (2001) Initial plasma HIV-1 Rna levels and progression to Aids in women and men. N Engl J Med 344:720–725

Stoiber H, Clivio A, Dierich MP (1997) Role of complement in HIV infection. Annu Rev Immunol 15:649–674

Straub RH (2007) The complex role of estrogens in inflammation. Endocr Rev 28:521–574

Stringer E, Antonsen E (2008) Hormonal contraception and HIV disease progression. Clin Infect Dis 47:945–951

Stringer EM, Giganti M, Carter RJ, El-Sadr W, Abrams EJ, Stringer JS (2009) Hormonal contraception and HIV disease progression: a multicountry cohort analysis of the Mtct-Plus Initiative. AIDS 23(Suppl 1):S69–S77

Stringer EM, Kaseba C, Levy J, Sinkala M, Goldenberg RL, Chi BH, Matongo I, Vermund SH, Mwanahamuntu M, Stringer JS (2007) A randomized trial of the intrauterine contraceptive device vs hormonal contraception in women who are infected with the human immunodeficiency virus. Am J Obstet Gynecol 197(144):e1–e8

Stringer JS, Zulu I, Levy J, Stringer EM, Mwango A, Chi BH, Mtonga V, Reid S, Cantrell RA, Bulterys M, Saag MS, Marlink RG, Mwinga A, Ellerbrock TV, Sinkala M (2006) Rapid scale-up of antiretroviral therapy at primary care sites in Zambia: feasibility and early outcomes. JAMA 296:782–793

Sulkowski MS, Mehta SH, Torbenson MS, Higgins Y, Brinkley SC, De Oca RM, Moore RD, Afdhal NH, Thomas DL (2007) Rapid fibrosis progression among HIV/hepatitis C virus-co-infected adults. AIDS 21:2209–2216

Sulkowski MS, Wasserman R, Brooks L, Ball L, Gish R (2004) Changes in haemoglobin during interferon alpha-2b plus ribavirin combination therapy for chronic hepatitis C virus infection. J Viral Hepat 11:243–250

Sullivan RJ, Pantanowitz L, Casper C, Stebbing J, Dezube BJ (2008) HIV/Aids: epidemiology, pathophysiology, and treatment of Kaposi sarcoma-associated herpesvirus disease: Kaposi sarcoma, primary effusion lymphoma, and multicentric Castleman disease. Clin Infect Dis 47:1209–1215

Suy A, Castro P, Nomdedeu M, Garcia F, Lopez A, Fumero E, Gallart T, Lopalco L, Coll O, Gatell JM, Plana M (2007) Immunological profile of heterosexual highly HIV-exposed uninfected individuals: predominant role of Cd4 and Cd8 T-cell activation. J Infect Dis 196: 1191–1201

Taha TE, Hoover DR, Dallabetta GA, Kumwenda NI, Mtimavalye LA, Yang LP, Liomba GN, Broadhead RL, Chiphangwi JD, Miotti PG (1998) Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. AIDS 12:1699–1706

Taha TE, Kumwenda NI, Gibbons A, Broadhead RL, Fiscus S, Lema V, Liomba G, Nkhoma C, Miotti PG, Hoover DR (2003) Short postexposure prophylaxis in newborn babies to reduce mother-to-child transmission of HIV-1: Nvaz randomised clinical trial. Lancet 362:1171–1177

Taha TE, Nour S, Kumwenda NI, Broadhead RL, Fiscus SA, Kafulafula G, Nkhoma C, Chen S, Hoover DR (2005) Gender differences in perinatal HIV acquisition among African infants. Pediatrics 115:e167–e172

Taneepanichskul S, Phuapradit W, Chaturachinda K (1997) Association of contraceptives and HIV-1 infection in Thai female commercial sex workers. Aust N Z J Obstet Gynaecol 37:86–88

Taylor-Smith K, Tweya H, Harries A, Schoutene E, Jahn A (2010) Gender differences in retention and survival on antiretroviral therapy of HIV-1 infected adults in Malawi. Malawi Med J 22: 49–56

Tebit DM, Ndembi N, Weinberg A, Quinones-Mateu ME (2012) Mucosal transmission of human immunodeficiency virus. Curr HIV Res 10:3–8

Temmerman M, Nyong'O AO, Bwayo J, Fransen K, Coppens M, Piot P (1995) Risk factors for mother-to-child transmission of human immunodeficiency virus-1 infection. Am J Obstet Gynecol 172:700–705

Thorne C, Newell ML (2004) Are girls more at risk of intrauterine-acquired HIV infection than boys? AIDS 18:344–347

Thorsteinsson K, Ladelund S, Jensen-Fangel S, Johansen IS, Katzenstein TL, Pedersen G, Storgaard M, Obel N, Lebech AM (2012) Impact of gender on response to highly active antiretroviral therapy in HIV-1 infected patients: a nationwide population-based cohort study. BMC Infect Dis 12:293

Tienen C, Van Der Loeff MS, Zaman SM, Vincent T, Sarge-Njie R, Peterson I, Leligdowicz A, Jaye A, Rowland-Jones S, Aaby P, Whittle H (2010) Two distinct epidemics: the rise of HIV-1 and decline of HIV-2 infection between 1990 and 2007 in rural Guinea-Bissau. J Acquir Immune Defic Syndr 53:640–647

Tollerud DJ, Clark JW, Brown LM, Neuland CY, Pankiw-Trost LK, Blattner WA, Hoover RN (1989) The influence of age, race, and gender on peripheral blood mononuclear-cell subsets in healthy nonsmokers. J Clin Immunol 9:214–222

Tomescu C, Duh FM, Lanier MA, Kapalko A, Mounzer KC, Martin MP, Carrington M, Metzger DS, Montaner LJ (2010) Increased plasmacytoid dendritic cell maturation and natural killer cell activation in HIV-1 exposed, uninfected intravenous drug users. AIDS 24:2151–2160

Trunova N, Tsai L, Tung S, Schneider E, Harouse J, Gettie A, Simon V, Blanchard J, Cheng-Mayer C (2006) Progestin-based contraceptive suppresses cellular immune responses in SHIV-infected rhesus macaques. Virology 352:169–177

Turner AN, Morrison CS, Padian NS, Kaufman JS, Salata RA, Chipato T, Mmiro FA, Mugerwa RD, Behets FM, Miller WC (2007) Men's circumcision status and women's risk of HIV acquisition in Zimbabwe and Uganda. AIDS 21:1779–1789

Ungchusak K, Rehle T, Thammapornpilap P, Spiegelman D, Brinkmann U, Siraprapasiri T (1996) Determinants of HIV infection among female commercial sex workers in northeastern Thailand: results from a longitudinal study. J Acquir Immune Defic Syndr Hum Retrovirol 12:500–507

Uphoff DE (1975) Comparative survival of lethally irradiated inbred male mice inoculated with marrow from virgin or multiparous female donors. J Natl Cancer Inst 54:1343–1348

Urbano-Ispizua A, Rozman C, Pimentel P, Solano C, De La Rubia J, Brunet S, Perez-Oteyza J, Ferra C, Zuazu J, Caballero D, Bargay J, Carvalhais A, Diez JL, Espigado I, Alegre A, Rovira M, Campilho F, Odriozola J, Sanz MA, Sierra J, Garcia-Conde J, Montserrat E (2002) Risk factors for acute graft-versus-host disease in patients undergoing transplantation with Cd34+ selected blood cells from Hla-identical siblings. Blood 100:724–727

Van Benthem BH, Vernazza P, Coutinho RA, Prins M (2002) The impact of pregnancy and menopause on Cd4 lymphocyte counts in HIV-infected women. AIDS 16:919–924

Van Den Berg CH, Grady BP, Schinkel J, Van De Laar T, Molenkamp R, Van Houdt R, Coutinho RA, Van Baarle D, Prins M (2011) Female sex and Il28B, a synergism for spontaneous viral clearance in hepatitis C virus (Hcv) seroconverters from a community-based cohort. PLoS One 6:e27555

Veazey RS, Marx PA, Lackner AA (2003a) Vaginal Cd4+ T cells express high levels of Ccr5 and are rapidly depleted in simian immunodeficiency virus infection. J Infect Dis 187:769–776

Veazey RS, Shattock RJ, Pope M, Kirijan JC, Jones J, Hu Q, Ketas T, Marx PA, Klasse PJ, Burton DR, Moore JP (2003b) Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. Nat Med 9:343–346

Verthelyi D (2006) Female's heightened immune status: estrogen, T cells, and inducible nitric oxide synthase in the balance. Endocrinology 147:659–661

Vishwanathan SA, Guenthner PC, Lin CY, Dobard C, Sharma S, Adams DR, Otten RA, Heneine W, Hendry RM, Mcnicholl JM, Kersh EN (2011) High susceptibility to repeated, low-dose, vaginal SHIV exposure late in the luteal phase of the menstrual cycle of pigtail macaques. J Acquir Immune Defic Syndr 57:261–264

Wald A, Link K (2002) Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: a meta-analysis. J Infect Dis 185:45–52

Wang CC, Mcclelland RS, Overbaugh J, Reilly M, Panteleeff DD, Mandaliya K, Chohan B, Lavreys L, Ndinya-Achola J, Kreiss JK (2004) The effect of hormonal contraception on genital tract shedding of HIV-1. AIDS 18:205–209

Wang CC, Mcclelland RS, Reilly M, Overbaugh J, Emery SR, Mandaliya K, Chohan B, Ndinya-Achola J, Bwayo J, Kreiss JK (2001) The effect of treatment of vaginal infections on shedding of human immunodeficiency virus type 1. J Infect Dis 183:1017–1022

Wang CC, Reilly M, Kreiss JK (1999) Risk of HIV infection in oral contraceptive pill users: a meta-analysis. J Acquir Immune Defic Syndr 21:51–58

Wang Y, Abel K, Lantz K, Krieg AM, Mcchesney MB, Miller CJ (2005) The Toll-like receptor 7 (Tlr7) agonist, imiquimod, and the Tlr9 agonist, CpG Odn, induce antiviral cytokines and chemokines but do not prevent vaginal transmission of simian immunodeficiency virus when applied intravaginally to rhesus macaques. J Virol 79:14355–14370

Watson-Jones D, Baisley K, Weiss HA, Tanton C, Changalucha J, Everett D, Chirwa T, Ross D, Clayton T, Hayes R (2009) Risk factors for HIV incidence in women participating in an Hsv suppressive treatment trial in Tanzania. AIDS 23:415–422

Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Li X, Laeyendecker O, Kiwanuka N, Kigozi G, Kiddugavu M, Lutalo T, Nalugoda F, Wabwire-Mangen F, Meehan MP, Quinn TC (2005) Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. J Infect Dis 191:1403–1409

Weber JN, Mccreaner A, Berrie E, Wadsworth J, Jeffries DJ, Pinching AJ, Harris JR (1986) Factors affecting seropositivity to human T cell lymphotropic virus type Iii (Htlv-Iii) or lymphadenopathy associated virus (Lav) and progression of disease in sexual partners of patients with Aids. Genitourin Med 62:177–180

Weber R, Ruppik M, Rickenbach M, Spoerri A, Furrer H, Battegay M, Cavassini M, Calmy A, Bernasconi E, Schmid P, Flepp M, Kowalska J, Ledergerber B (2013) Decreasing mortality and changing patterns of causes of death in the Swiss HIV Cohort Study. HIV Med 14:195–207

Weiser SD, Leiter K, Bangsberg DR, Butler LM, Percy-De Korte F, Hlanze Z, Phaladze N, Iacopino V, Heisler M (2007) Food insufficiency is associated with high-risk sexual behavior among women in Botswana and Swaziland. PLoS Med 4:1589–1597, Discussion 1598

Weiss HA, Buve A, Robinson NJ, Van Dyck E, Kahindo M, Anagonou S, Musonda R, Zekeng L, Morison L, Carael M, Laga M, Hayes RJ (2001) The epidemiology of Hsv-2 infection and its association with HIV infection in four urban African populations. AIDS 15(Suppl 4):S97–S108

Weiss HA, Quigley MA, Hayes RJ (2000) Male circumcision and risk of HIV infection in sub-Saharan Africa: a systematic review and meta-analysis. AIDS 14:2361–2370

Weisser M, Rudin C, Battegay M, Pfluger D, Kully C, Egger M (1998) Does pregnancy influence the course of HIV infection? Evidence from two large Swiss cohort studies. J Acquir Immune Defic Syndr Hum Retrovirol 17:404–410

White HD, Musey LK, Andrews MM, Yeaman GR, Demars LR, Manganiello PD, Howell AL, Wira CR, Green WR, Mcelrath MJ (2001) Human immunodeficiency virus-specific and Cd3-redirected cytotoxic T lymphocyte activity in the human female reproductive tract: lack of correlation between mucosa and peripheral blood. J Infect Dis 183:977–983

Wieser F, Hosmann J, Tschugguel W, Czerwenka K, Sedivy R, Huber JC (2001) Progesterone increases the number of Langerhans cells in human vaginal epithelium. Fertil Steril 75: 1234–1235

Williams PL, Abzug MJ, Jacobson DL, Wang J, Van Dyke RB, Hazra R, Patel K, Dimeglio LA, Mcfarland EJ, Silio M, Borkowsky W, Seage GR 3rd, Oleske JM, Geffner ME (2013) Pubertal onset in children with perinatal HIV infection in the era of combination antiretroviral treatment. AIDS 27:1959–1970

Wira CR, Fahey JV, Ghosh M, Patel MV, Hickey DK, Ochiel DO (2010) Sex hormone regulation of innate immunity in the female reproductive tract: the role of epithelial cells in balancing reproductive potential with protection against sexually transmitted pathogens. Am J Reprod Immunol 63:544–565

Wolfs TF, Zwart G, Bakker M, Goudsmit J (1992) HIV-1 genomic RNA diversification following sexual and parenteral virus transmission. Virology 189:103–110

Wools-Kaloustian K, Kimaiyo S, Diero L, Siika A, Sidle J, Yiannoutsos CT, Musick B, Einterz R, Fife KH, Tierney WM (2006) Viability and effectiveness of large-scale HIV treatment initiatives in sub-Saharan Africa: experience from western Kenya. AIDS 20:41–48

Wyle FA, Kent JR (1977) Immunosuppression by sex steroid hormones. The effect upon PHA- and PPD-stimulated lymphocytes. Clin Exp Immunol 27:407–415

Yan N, Chen ZJ (2012) Intrinsic antiviral immunity. Nat Immunol 13:214–222

Yeaman GR, Howell AL, Weldon S, Demian DJ, Collins JE, O'connell DM, Asin SN, Wira CR, Fanger MW (2003) Human immunodeficiency virus receptor and coreceptor expression on human uterine epithelial cells: regulation of expression during the menstrual cycle and implications for human immunodeficiency virus infection. Immunology 109:137–146

Yin MT, Zhang CA, Mcmahon DJ, Ferris DC, Irani D, Colon I, Cremers S, Shane E (2012) Higher rates of bone loss in postmenopausal HIV-infected women: a longitudinal study. J Clin Endocrinol Metab 97:554–562

Zang YC, Halder JB, Hong J, Rivera VM, Zhang JZ (2002) Regulatory effects of estriol on T cell migration and cytokine profile: inhibition of transcription factor Nf-kappa B. J Neuroimmunol 124:106–114

Zaragoza-Macias E, Cosco D, Nguyen ML, Del Rio C, Lennox J (2010) Predictors of success with highly active antiretroviral therapy in an antiretroviral-naive urban population. AIDS Res Hum Retroviruses 26:133–138

Zeitler PS, Travers S, Kappy MS (1999) Advances in the recognition and treatment of endocrine complications in children with chronic illness. Adv Pediatr 46:101–149

Zhang J, Li G, Bafica A, Pantelic M, Zhang P, Broxmeyer H, Liu Y, Wetzler L, He JJ, Chen T (2005) Neisseria gonorrhoeae enhances infection of dendritic cells by HIV type 1. J Immunol 174:7995–8002

Zhang LQ, Mackenzie P, Cleland A, Holmes EC, Brown AJ, Simmonds P (1993) Selection for specific sequences in the external envelope protein of human immunodeficiency virus type 1 upon primary infection. J Virol 67:3345–3356

Zhang Z, Schuler T, Zupancic M, Wietgrefe S, Staskus KA, Reimann KA, Reinhart TA, Rogan M, Cavert W, Miller CJ, Veazey RS, Notermans D, Little S, Danner SA, Richman DD, Havlir D, Wong J, Jordan HL, Schacker TW, Racz P, Tenner-Racz K, Letvin NL, Wolinsky S, Haase AT (1999) Sexual transmission and propagation of SIV and HIV in resting and activated Cd4+ T cells. Science 286:1353–1357

Zhang ZQ, Wietgrefe SW, Li Q, Shore MD, Duan L, Reilly C, Lifson JD, Haase AT (2004) Roles of substrate availability and infection of resting and activated Cd4+ T cells in transmission and acute simian immunodeficiency virus infection. Proc Natl Acad Sci U S A 101: 5640–5645

Zhu J, Hladik F, Woodward A, Klock A, Peng T, Johnston C, Remington M, Magaret A, Koelle DM, Wald A, Corey L (2009) Persistence of HIV-1 receptor-positive cells after Hsv-2 reactivation is a potential mechanism for increased HIV-1 acquisition. Nat Med 15: 886–892

Zhu T, Mo H, Wang N, Nam DS, Cao Y, Koup RA, Ho DD (1993) Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science 261:1179–1181

Zorrilla EP, Mckay JR, Luborsky L, Schmidt K (1996) Relation of stressors and depressive symptoms to clinical progression of viral illness. Am J Psychiatry 153:626–635

Zulu EM, Dodoo FN, Chika-Ezee A (2002) Sexual risk-taking in the slums of Nairobi, Kenya, 1993–8. Popul Stud (Camb) 56:311–323

Chapter 6
Sex Differences in Influenza Virus Infection, Vaccination, and Therapies

Jackye Peretz, Olivia J. Hall, and Sabra L. Klein

Abstract Males and females differ in the likely outcome of influenza virus infection and vaccination. Following infection with pandemic and outbreak strains of influenza viruses, females of reproductive ages (15–49 years of age) experience a worse outcome than their male counterparts. Among females of reproductive ages, pregnancy is one factor linked to an increased risk of severe outcome of influenza, although it is not the sole factor explaining the female preponderance of severe disease. The sex bias in disease outcome is reversed in children under the age of 10 years and adults over the age of 65 years, where males appear to be more likely to be hospitalized or die from influenza. Small animal models of influenza virus infection illustrate that inflammatory immune responses also differ between the sexes and impact the outcome of infection. Males and females also generally respond differently to influenza vaccines and antiviral treatments, with females on average initiating higher humoral immune responses following vaccination and experiencing more adverse reactions to vaccines and drug treatments than males. Small animal models further show that elevated immunity following vaccination in females compared with males leads to greater cross protection against novel influenza viruses. We hypothesize that sex steroid hormones, including estrogens, progesterone, and androgens, as well as genetic differences between the sexes, may play roles in modulating sex differences in immune responses to influenza virus infection and vaccination.

6.1 Introduction

The prevalence of viral infections, including influenza, differs between male and female humans (Klein 2012). Behavioral factors, including occupation, personal hygiene (e.g., hand washing), and familial responsibilities (e.g., caring for children or the elderly), can influence exposure to viruses. Several studies further illustrate

J. Peretz • O.J. Hall • S.L. Klein (✉)
The W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
e-mail: sklein2@jhu.edu

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_6

that physiological differences between males and females can lead to differential responses to infection and, therefore, outcomes. Females often display reduced susceptibility to viral infections because they mount stronger immune responses than males. The innate recognition and response to viruses as well as downstream adaptive immune responses differ between males and females during viral infections (Markle and Fish 2014). As a result of heightened immunity to viruses, both the intensity (i.e., viral load within an individual) and prevalence (i.e., number of infected individuals within a population) of viral infections are often lower for females than males (Klein 2012). Much of the disease attributed to viral infection results from aberrant host inflammatory responses (Meier et al. 2009; Robinson et al. 2011b). Consequently, heightened antiviral, inflammatory, and cellular immune responses in females relative to males, though essential for virus clearance, may underlie increased symptoms of disease among females as compared with males following infection. Sex differences in immune responses to viral infections may further depend on age-related factors and differ prior to puberty, after puberty, during pregnancy, or after reproductive senescence.

Sex and gender differences also are apparent in the responses and efficacy of prophylaxis and therapeutic treatments for viral diseases, including influenza. Vaccines are the principal preventative treatment for viral diseases and have successfully reduced many diseases in both males and females. The efficacy of vaccines relies on their ability to induce protective immunity, at least in the short-term for influenza viruses. There is growing evidence that both protective immune responses and adverse reactions to viral vaccines, including FDA-approved influenza vaccines, are higher in females than males (Klein et al. 2010a). Most antiviral drugs are used as a therapeutic treatment for specific viral infections, with these drugs typically inhibiting the replication of viruses in host cells. Only recently has it been documented that the pharmacokinetics (i.e., absorption, distribution, metabolism, and excretion of drugs) and pharmacodynamics (i.e., the effect of the drug on physiological and biochemical processes, both therapeutic and adverse) differ between the sexes (see Chapt. 4) (Klein 2012). Using data available for influenza virus infection, vaccination, and antiviral treatments, we will: (1) address age-specific sex differences in the outcome of influenza virus infections; (2) evaluate how the efficacy of vaccines and antiviral treatments for influenza differ between the sexes; (3) explore the female-specific state of pregnancy and the impact of pregnancy on the outcome of influenza virus infection and vaccination; and (4) identify possible hormonal and genetic mechanisms that contribute to immunological differences between males and females in response to influenza viruses and vaccines. The contribution of gender-related differences in exposure to influenza viruses as well as access to, compliance with, and acceptance of treatments for influenza will be addressed where data exist; the primary focus throughout this chapter, however, will be on the biological differences between males and females. All too often sex differences are either ignored or understudied in clinical and basic biomedical research. As a result, significant gaps exist in our understanding of how biological differences between males and females affect the efficacy of treatments for influenza. We are encouraged by the recent recommendation by the

World Health Organization (WHO) indicating that information about sex and gender should be provided when reporting influenza infections (WHO 2014b).

6.2 Basic Influenza Biology

Influenza is a perpetually reemerging disease because of the continual genetic evolution of influenza A viruses (Subbarao et al. 2006). Typically, three strains of influenza viruses, two influenza A viruses and one influenza B virus, circulate in a given year and are largely responsible for influenza outbreaks, pandemics, and epidemics. While there are no differing antigenic subtypes of influenza B, influenza A viruses are a constant concern because they continually undergo antigenic drift and, less frequently, antigenic shift. The WHO monitors these genetic changes in influenza A viruses to keep seasonal influenza vaccines relevant to the current circulating antigenic subtypes of influenza A (Subbarao et al. 2006). Antigenic subtype is determined by the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). There are currently sixteen possible HA proteins and nine NA proteins, forming the strain identification of each virus (e.g., H1N1, H3N2, and H5N1) (Medina and Garcia-Sastre 2011). Currently, the A/H3N2 and A/H1N12009 pandemic (pdm) strain co-circulate with influenza B (CDC 2014c, d). These three strains form the basis for the annual vaccines that induce protective antibody responses against the HA proteins of an A/H1N1, A/H3N2, and influenza B virus. In the event of an outbreak (e.g., H5N1) or pandemic due to antigenic shift, such as with the 2009 H1N1pdm strain, a monovalent vaccine can be developed that specifically targets a single HA protein on the new strain of influenza A virus.

Several laboratory animal models are used in preclinical influenza research. Some animals, such as ferrets and guinea pigs, are naturally susceptible to human influenza viruses and can readily transmit the viruses (Bouvier and Lowen 2010). Mice are the most widely utilized animal model for influenza research. There are numerous advantages and disadvantages to using mice as models of human diseases. For our purposes, mouse models recapitulate the sex differences seen in human populations in the pathogenesis of influenza virus infection and vaccination and provide us with a system for identifying the underlying mechanisms. One drawback is that mice are not naturally susceptible to human influenza viruses, so the viruses must be adapted through serial passages in mice. The most widely used models involve the use of BALB/c or C57BL/6 strains infected with mouse-adapted (ma) influenza strains (e.g., A/Puerto Rico/8/1934) (Bouvier and Lowen 2010).

6.3 Sex Differences in Influenza Virus Infection

6.3.1 Seasonal Influenza Epidemics

Seasonal influenza infection results in an acute, self-limiting infection and, as a consequence, most cases are unreported. Available data on differences in the number of cases of seasonal influenza between males and females worldwide are limited, and, therefore, conclusions are difficult to draw. Most case reports of seasonal influenza do not analyze data for male–female differences, or if they do, then they often do not consider the interaction between sex and age. Examination of the limited available reported seasonal influenza cases that required hospitalization reveals that the severity of infection is higher in prepubertal and elderly males compared with age-matched females (Quach et al. 2003; Crighton et al. 2004, 2008). Data from Denmark suggest that male–female differences in the risk of hospitalization from seasonal influenza virus shift at puberty. Thus, males are more likely to have severe seasonal influenza illness before puberty, whereas females are more likely to have severe seasonal influenza illness after puberty and before menopause (Jensen-Fangel et al. 2004).

6.3.2 Outbreaks of Avian H5N1 in Eurasia

Avian H5N1 is a highly pathogenic influenza virus that affects the lower respiratory tract in humans and is primarily transmitted from diseased poultry to humans, with rare person-to-person transmission (CDC 2014b; Ng and To 2007). From 2003 through January 2014, of the 739 cases of confirmed (650) and suspected (89) H5N1 infections that have been reported worldwide, 592 of those cases resulted in death (~80 % fatality) (WHO 2014d). Worldwide, the incidence and severity of H5N1 infection and mortality induced by H5N1 infection is greater among young adult females (10–39 years of age) than males (WHO 2013) (Table 6.1). Between 2004 and 2006, there were no male–female differences reported for any of these parameters in either Vietnam or China (Yu et al. 2008; Liem et al. 2009; Hien et al. 2009). Conversely, in Indonesia, from 2005 to 2006, the case fatality rate was significantly higher for females (90 %) compared with males (67 %) (Sedyaningsih et al. 2007). Similarly, in Egypt from 2009 to 2010, young adult females (>10 years old) comprised on average 60 % of H5N1 cases and 90 % of associated fatalities. The incidence of H5N1 was found to be higher in males than females among individuals <10 years of age (Kayali et al. 2011; Dudley 2009; Arima and Vong 2013). The annual, as well as country, variation in the male–female differences suggest that gender-related factors, including occupational exposure, play a significant role. For example, while men are more likely to slaughter poultry, females have more direct exposure to poultry as they are more likely to boil poultry, feed and care for backyard poultry, and purchase poultry in

Table 6.1 Sex differences in the outcome of influenza virus infection in humans

Classification	Influenza A virus	Sex difference	Age (years)	Dependent measure	References
Outbreak	H5N1	F > M	Total	Incidence	Arima and Vong (2013), Kayali et al. (2011), Dudley and Mackay (2013), CDC (2014b)
		M > F	<10	Incidence	
		F > M	10–39	Severity[a]/ Mortality	
		M > F	>39	Severity/ Mortality	
	H7N9	M > F M > F	Total >45	Incidence, Severity, Mortality	WHO (2014c), Li et al. (2014), Dudley and Mackay (2013)
Pandemic	1918 H1N1pdm	M > F	Total	Incidence, Severity, Mortality	Noymer and Garenne (2000)
		M > F	20–40		
	1957 H2N2pdm	M = F	Total	Mortality	Serfling et al. (1967), Kilbourne (2006)
		F > M	1–44		
		M > F	>44		
	2009 H1N1pdm	F > M	Total	Incidence	WHO (2014a, b), Ontario (2009), CDC (2014c), Kumar et al. (2009)
		M > F	<18	Incidence/ Severity	
		F > M	18–65	Incidence/ Severity	
		M > F	>65	Severity	

[a]Severity = hospitalization with severe disease

markets (Fasina et al. 2010). Two studies of poultry workers, however, suggest that direct exposure to poultry does not fully explain the risk of H5N1 infection and that other variables should be considered to explain the male–female differences, such as coinfection with another virus (Fasina et al. 2010; Briand and Fukuda 2009). Coinfection with other pathogens is hypothesized to be one factor mediating increased susceptibility to avian influenza viruses. During the 2009–2010 influenza season, young adult females were at a greater risk of 2009 H1N1pdm infection compared with age-matched males which may have also placed them at a higher risk of coinfection with H5N1 (Fasina et al. 2010). Further investigation into the potential antibody-dependent enhancement of related influenza strains is warranted to uncover possible biological explanations for the increased susceptibility to H5N1infection among young adult females compared with males of similar age.

6.3.3 Outbreak of Avian H7N9 in China

In Spring 2013, an outbreak of avian H7N9 influenza was detected in humans, primarily in China, with one confirmed case in Malaysia (CDC 2014a). Avian H7N9 influenza virus originates from diseased poultry and migratory birds and is

not currently transmissible from person to person (Yang et al. 2013). Since the Spring of 2013, 136 cases of H7N9 have been reported throughout China, with 44 deaths from the infection (WHO 2014c). Overall, old males (>50 years of age) are at the greatest risk for contracting H7N9 infection, comprising approximately two-thirds of the cases of H7N9 infection (WHO 2014c; Skowronski et al. 2013) (Table 6.1). Data from China indicate an overall case fatality rate of 32–34 % (Li et al. 2014; Dudley and Mackay 2013; Arima and Vong 2013), with higher fatality rates among old males than either age-matched females or young (<50 years of age) males and females. Surveillance information gathered by the Chinese Center for Disease Control as well as localized surveillance studies for 2013 through February 2014 indicate that 62–71 % of all H7N9 cases were male, with 75 % of confirmed H7N9 cases being males over 45 years of age and 74 % of all fatalities being males (Wang et al. 2014; Li et al. 2014; Dudley and Mackay 2013; Cowling et al. 2013; Arima and Vong 2013). Therefore, both male sex and older age are risk factors for infection with and morality from H7N9 influenza virus. Whether older males are more likely to come in contact with diseased poultry or migratory birds has not been shown. There are few studies that provide mechanistic explanations for the male–female differences. However, in one study, elevated antibody responses against H7N9 were present in a majority of nonfatal cases of H7N9 infection, but in only one-fourth of fatal cases, suggesting a failure of the humoral immune response may contribute to severe outcome of disease (Yang et al. 2014). Aging and male sex are independently associated with reduced humoral immune responses (see below) and may account for the increased susceptibility to and number of fatalities observed in old males with H7N9 infection.

6.3.4 Worldwide Influenza Pandemics

Historically, very little information exists to explain male–female differences in the outcome of pandemic influenza virus infection. The 1918 H1N1 influenza pandemic was the most deadly influenza pandemic to date, causing 20–100 million deaths worldwide (Noymer and Garenne 2000). This influenza pandemic was disproportionately fatal in young adult males (20–40 years of age; median 28 years) and was exacerbated by coinfection with tuberculosis, which is also considered to be a male-dominant disease (Morens et al. 2008; Kilbourne 2006; Gagnon et al. 2013) (see Chap. 8).

Unlike the 1918 H1N1 pandemic, the 1957 H2N2 pandemic was the first pandemic that was lethal without a secondary bacterial infection. The 1957 H2N2 pandemic resulted in higher fatality rates among females than males (<50 years of age), despite the widespread use of vaccine therapy (Serfling et al. 1967; Kilbourne 2006) (Table 6.1). Most of the fatal cases of H2N2 pandemic had underlying cardiac or pulmonary conditions. Cardiopulmonary diseases, in general, can be more frequent and severe in both pregnant and nonpregnant females than males (Klein et al. 2010b; Neuzil et al. 1998); thus, sex-biased comorbid conditions may

have contributed to the increased rates of severe disease and mortality among young adult females during the 1957 H2N2 pandemic (Kilbourne 2006).

During the 2009 H1N1 pandemic in the United States, while females were more likely to develop severe disease than males (53.2 % female vs. 46.8 % male hospitalizations), male–female differences in the incidence, severity of 2009 H1N1pdm infection, and mortality rates due to it were dependent on age at the time of infection (CDC 2014c). Among individuals less than 19 years of age, males were more likely to contract 2009 H1N1pdm virus and suffer more severe illness, whereas in adults aged 19–64 years, the bias was reversed. Among adults, females were at a higher risk of hospitalization and death from 2009 H1N1pdm infection than males (Jacobs et al. 2012). Among older individuals (i.e., 75 years of age and older), males were at a higher risk of hospitalization, but females were at a higher risk of mortality (Jacobs et al. 2012) (Table 6.1).

In Canada, during the first wave of the pandemic (April–August 2009), there were 168 critically ill patients with confirmed or probable 2009 H1N1 influenza, a majority of which were young adult females (67.3 % with 7.7 % pregnant) (Kumar et al. 2009). Additional data from Canada (April–May 2009) indicated that male–female differences in the incidence of infection varied with age, such that the incidence of infection with 2009 H1N1pdm was higher in males than females at 10–19 years of age, higher in females than males 20–39 years of age, and equivalent between the sexes after 40 years of age (Ontario 2009). The reason for the greater proportion of hospitalized adult females than males in Canada is not known, but many cases involved comorbid conditions, including chronic lung disease (e.g., asthma), which is typically more severe in males prior to puberty and females during young adulthood (Singh et al. 1999; Schatz et al. 2006; Schatz and Camargo 2003; Ontario 2009; Moorman and Rudd 2007).

In Brazil (April–August 2009), rates of hospitalization with severe acute respiratory illness were higher among females (57.5 %) than males, with a majority of the females being of reproductive age (15–49 years of age), of which 20.8 % were pregnant (Ontario 2009; Oliveira et al. 2009). In Portugal, 82 % of deaths from the 2009 H1N1pdm virus were females over 75 years of age (Nogueira et al. 2009). Finally, in Japan, data from the 2009 H1N1 pandemic as well as from 2005, which was a particularly severe seasonal influenza year, revealed profound sex differences in morbidity rates (Eshima et al. 2011). At younger (<20 year of age) and older (>80 years of age) ages, the morbidity rates were higher for males than females. Conversely, during the reproductive years (20–49 years of age), morbidity rates were higher for females than males. Most countries, including the United States, did not stratify and analyze the 2009 H1N1 pandemic data by age and sex. Analysis of male–female differences in incidence data stratified by both sex and age group is necessary to properly evaluate whether these differences are conserved across the life span and in diverse regions of the world. The Canadian government has led the way by requiring disaggregation and analysis of data by sex, which is the most parsimonious explanation for why male–female differences were so widely reported in Canada, and not in other countries, including the United States, during the 2009 H1N1 pandemic.

6.3.5 *Animal Models*

Small animal models have been instrumental in characterizing sex differences in the outcome of influenza virus infection and in determining some of the mechanisms mediating these differences (Robinson et al. 2011a, b; Larcombe et al. 2011). We have established that male mice are more resistant to influenza viruses than females. When adult male and female C57BL/6 mice are inoculated with ma H1N1 (i.e., A/Puerto Rico/8/1934 [A/PR8]) or H3N2 (i.e., A/Hong Kong/68 [A/HK68]) viruses using 5 $\log_{10}$ dilutions to determine the median lethal dose (LD_{50}) for each sex, the LD_{50} for females is 11-fold lower for A/PR8 and 4-fold lower for A/HK68 than the LD_{50} for males (Lorenzo et al. 2011). Sex differences in morbidity following infection with H1N1 or H3N2 viruses are also dose-dependent with females experiencing greater body mass loss and hypothermia than males after infection with median doses. In contrast, at sublethal doses, both sexes experience minor, transient morbidity, and at high, lethal doses, both sexes experience extreme morbidity (Lorenzo et al. 2011). Thus, females can experience a worse outcome following infection with A/PR8 and A/HK68 viruses, but this effect is dose dependent.

Female mice consistently show greater reductions in body mass and body temperature as well as survival as compared with males when infected with median doses of A/PR8 (Robinson et al. 2011b). Titers of infectious virus in the lungs do not differ between the sexes, suggesting that changes in virus load alone are not responsible for the observed sex differences in morbidity and mortality. Highly pathogenic influenza viruses cause severe disease by initiating profound pro-inflammatory cytokine and chemokine responses (Guan et al. 2004; de Jong et al. 2006). Consequently, within the first week after infection with A/PR8, females show a greater induction of cytokines and chemokines, including CCL2, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and interleukin-6 (IL-6,) in their lungs than males. Similarly, female adult BALB/c mice infected with a mouse-adapted H3N1 influenza virus develop greater lung hyperresponsiveness to methacholine challenge and produce more CCL2 than male mice (Larcombe et al. 2011). However, as no differences in virus titers are evident between the sexes, host-mediated immunopathology rather than virus replication is hypothesized to underlie sex differences in influenza pathogenesis.

6.4 Sex Differences in Responses to Influenza Vaccines and Antiviral Treatments

6.4.1 *Acceptance and Receipt of Vaccines*

Acceptance of (i.e., the intention of receiving) influenza vaccines is passively measured through questionnaires. The intention of receiving either pandemic or

avian influenza vaccines is reportedly 2–3 times lower for females than males, even among health care providers (e.g., nurses and general practitioners) in the United States, France, and Hong Kong (Opstelten et al. 2008; Ailes et al. 2013; Chor et al. 2009; Beau et al. 2014). Receipt of seasonal trivalent inactivated influenza vaccines (TIV) in the United States and in several European countries is consistently lower among both young and old adult females than their male counterparts (Endrich et al. 2009; Merrill and Beard 2009; Bean-Mayberry et al. 2009; Jimenez-Garcia et al. 2010). A systematic review revealed that during the 2009 H1N1 pandemic, receipt of the monovalent vaccine was consistently higher in males than females worldwide (Furman et al. 2013). Because male-biased acceptance and receipt of influenza vaccines is observed among old adults (i.e., postmenopausal), as well as young adults, pregnant women refusing vaccination cannot solely be responsible for the lack of acceptance and receipt of influenza vaccines among females.

6.4.2 Antibody Responses to Vaccines

Among both young (18–64 years of age) and old (65+ years of age) adults, females have higher hemagglutination inhibition (HAI) antibody titers than males following TIV administration. Receipt of either a full or half dose of seasonal TIV in adults 18–49 years of age results in HAI antibody titers that are at least twice as high in females than males (Engler et al. 2008). Following vaccination, adult females develop higher HAI and neutralizing antibody titers than adult males against H1N1, H3N2, and influenza B antigens (Engler et al. 2008; Furman et al. 2013). Among old adults that received the standard intramuscular seasonal TIV, higher HAI titers are associated with lower rates of hospitalization and mortality in females than in males. This suggests that the efficacy of the TIV in old adults may be higher for females (Wang et al. 2002; Chan et al. 2010). Among old adults, antibody responses to high-dose TIV are consistently higher than responses to standard-dose TIV for both males and females. Sex differences in HAI titers to high-dose TIV are still apparent, with antibody responses against each of the three influenza antigens being significantly higher in old females compared with age-matched males (Falsey et al. 2009).

Whether sex differences occur in response to seasonal live attenuated influenza vaccine (LAIV) has not been reported. Similar to seasonal TIV administration, old females were reported to have higher HAI antibody titers against the monovalent 2009 H1N1pdm inactivated vaccine than males, resulting in a 2–3 times higher seroprotection and seroconversion rate in females than males (Chudasama et al. 2013). Although older females produced higher antibody responses to the H1N1pdm vaccine, the avidity of their antibodies after H1N1pdm vaccination was significantly lower than that of old males (Khurana et al. 2012). If higher avidity is a measure of a superior antibody response in the elderly, then these data suggest that

the quality of the antibody response may be better for males than females. Conversely, cross-reactivity of antibody may be higher for females than males.

Animal models often provide insights into differential efficacy of vaccines. When immunized with a ma H1N1 (i.e., A/PR8) or H3N2 (i.e., A/HK68) influenza virus, adult female mice of reproductive ages mount higher neutralizing and total antibody responses than males (Lorenzo et al. 2011). Following vaccination, female mice are better protected against lethal challenge with a novel influenza strain than males (Lorenzo et al. 2011). Although elevated immunity afforded females greater cross protection than males against lethal challenge with novel influenza viruses, both sexes are equally protected against lethal challenge with homologous virus (i.e., the strain of virus in the vaccine) (Lorenzo et al. 2011).

6.4.3 Adverse Reactions to Vaccines

Passive (i.e., voluntary) reporting of local reactions (e.g., muscle pain, redness, and inflammation) to influenza vaccines is more frequent for females than males among both young and old adults (Cook 2009). Measurements of local erythema and induration, both of which are associated with inflammation, reveal that both young and old adult females have larger ($\geq$6 mm) injection site reactions to TIV than their male counterparts (Cate et al. 1983). Systemic reactions (e.g., fever, chills, nausea, headaches, and body aches) to TIV also are more commonly reported by females than males, with fatigue and headache being the most notable systemic reactions that occur more frequently in adult females than males (Nichol et al. 1996). Reports of local and systemic adverse reactions also are more frequent among adult females than males following receipt of the inactivated monovalent 2009 H1N1pdm vaccine (Elenkov et al. 2001; Fofie et al. 2005). The types of adverse reactions to the 2009 H1N1pdm vaccine that were reported, however, were similar between the sexes (Elenkov et al. 2001). To date, whether altering the dose or route of administration for the vaccine could reduce adverse reactions in females has not been analyzed.

A higher frequency of adverse reactions following receipt of the MF59-adjuvanted monovalent H5N1 vaccine was reported in females than males (Gao et al. 2013). When administered alone either intramuscularly or intradermally, the adjuvant aluminum hydroxide, which has been used as an adjuvant for influenza vaccines, causes greater injection site reactions in adult females than males (Pittman 2002). A recent analysis of passive reports to the Vaccine Adverse Event Reporting System revealed that immediate hypersensitivity reactions were higher in females than males (10–69 years of age) following receipt of either inactivated or live attenuated monovalent H1N1pdm vaccines (Griffioen and Halsey 2014).

6.4.4 Responses to Antiviral Treatments

Following infection, neuraminidase inhibitors can be administered to alleviate symptoms of disease and virus shedding (De Clercq 2006). Oseltamivir (Tamiflu) is administered orally, absorbed in the gastrointestinal tract, and converted to the active metabolite, oseltamivir carboxylate, by an esterase in the liver (De Clercq 2006). Zanamivir (Relenza) is an inhaled powder delivered as the active compound directly into the respiratory tract (De Clercq 2006). In patients with confirmed influenza A virus infection and treated with oseltamivir, alleviation of symptoms of disease is faster, and the reduction of nasal virus load is greater among males than females (Blanchon et al. 2011). In contrast, in influenza A virus-infected patients treated with zanamivir, no sex differences in either alleviation of symptoms or virus load are observed, suggesting that male–female differences in drug absorption or metabolism may contribute to the dimorphic outcome of treatment with oseltamivir but not zanamivir (Blanchon et al. 2011). Data also suggest that, at least in newborns, females clear oseltamivir more rapidly than males (Maltezou et al. 2011). Male–female differences in the outcome of oseltamivir treatment do not appear to be due to differences in treatment compliance (Blanchon et al. 2011). Future clinical studies must continue to partition and analyze antiviral drug outcome data by sex and establish the biological mechanisms mediating how oseltamivir is more effective in males than females.

6.5 Effects of Pregnancy on Influenza Pathogenesis and Responses to Vaccine

6.5.1 Infection

Pregnancy is a female-specific risk factor for severe disease following infection with seasonal influenza and was for the 1918 H1N1, 1957 H2N2, 1968 H3N2, and 2009 H1N1 pandemic viruses (Beigi 2012; Memoli et al. 2013; Liu et al. 2013) (Table 6.2). Pregnant women are 3 to 10 times more likely to be hospitalized and are at greater risk of intensive care unit (ICU) admission and secondary bacterial infections as compared to age-matched nonpregnant females (Jamieson et al. 2009; Klein et al. 2010b; Pazos et al. 2012a). These outcomes are less well characterized for avian influenza viruses, but the few cases of infected pregnant women reported indicate an increased risk of a severe disease outcome (Shu et al. 2006; Qi et al. 2014).

During the 1918 H1N1 pandemic, the fatality rate for pregnant women was over 50 % (vs. 33 % in the general population). Similar to young males, the increased fatality rate from the 1918 H1N1pdm virus among pregnant women was associated with secondary bacterial infections (Woolston and Conley 1918; Harris 1919) (Table 6.2). During the 2009 H1N1 pandemic, pregnant women were also at greater risk of a severe outcome, with approximately 80 % of maternal deaths occurring

Table 6.2 The effects of pregnancy on the outcome of influenza virus infection in humans

Influenza virus	Pregnancy effect	Dependent measure	References
1918 H1N1pdm	P > GP	Mortality	Woolston and Conley (1918), Harris (1919)
	P > GP	Secondary bacterial infection	
1957 H2N2pdm	P > GP	Incidence and risk of abortion	Hardy et al. (1961)
2009 H1N1pdm	$P_3 > P_1$ and P > GP	Incidence	Louie (2009), Jamieson et al. (2009), Jain et al. (2009), Creanga et al. (2011) Doyle et al. (2013) Rojas-Suarez et al. (2014)
	P > NP	Hospitalizations	
	P_2-P_3 > GP	ICU admissions	
	P_3 > NP	Mortality	
Seasonal influenza	P > NP	Incidence	Griffiths et al. (1980), Neuzil et al. (1998), Cox et al. (2006), Rogers et al. (2010)
	P > GP	Hospitalizations	
	P_3 > NP	ICU admissions	
	P_3 > NP	Complications	

P pregnant, P_1 pregnant first trimester, P_2 pregnant second trimester, P_3 pregnant third trimester, *GP* general population, *NP* nonpregnant women

during the third trimester of pregnancy (WHO 2009; Jain et al. 2009; Vaillant et al. 2009; Rojas-Suarez et al. 2014). Although the outcome of seasonal influenza virus infection is less severe than the outcome of pandemic influenza viruses, pregnant women have a longer length of hospitalization from infection with seasonal influenza viruses (Cox et al. 2006) (Table 6.2). Moreover, the risk of severe influenza increases during the course of pregnancy, with rates of hospitalization being greatest during the third trimester (Griffiths et al. 1980; Neuzil et al. 1998; Rogers et al. 2010) (Table 6.2).

Pregnant women may be more susceptible to influenza viruses due to changes in their immune status (see Chap. 3). During the course of pregnancy, the local and systemic immunological environment becomes less inflammatory in order to sustain a healthy pregnancy and avoid rejection of the fetus. Pregnancy in both humans as well as murine models is associated with a decrease in pro-inflammatory responses and a shift toward regulatory and anti-inflammatory responses, characterized by an increase in CD4+ T helper type 2 (Th2) cells and CD4+ T regulatory (Treg) cells (Fofie et al. 2005; Elenkov et al. 2001; Rowe et al. 2012; Marzi et al. 1996; Kraus et al. 2010). These changes in the immunological profile during pregnancy parallel increases in estrogens and progesterone over the three trimesters of pregnancy (Robinson and Klein 2012). Finally, anatomical and physiological changes over the course of pregnancy increase cardiopulmonary demands (Louie et al. 2010; Mosby et al. 2011; Klein et al. 2010b). The hormonal, immunological, physiological, and anatomical changes that occur during pregnancy may all contribute to an increased risk of a severe outcome from influenza virus infection.

Very little is known about the immunological responses to influenza viruses during pregnancy. Following 2009 H1N1pdm virus infection, pregnant women had decreased numbers of plasmacytoid dendritic cells (pDCs). These cells expressed an altered phenotype that lead to a downregulation of the activation of cytotoxic CD8+ T cells and their production of the pro-inflammatory cytokine IFN-γ (Vanders et al. 2013). These changes in CD8+ T cell activity during pregnancy have also been demonstrated in a murine model of pregnant mice infected with an maH1N1 strain (Pazos et al. 2012b). Pregnant mice infected with ma2009 H1N1 virus have an increase in numbers of pulmonary macrophages and Treg cells as compared with nonpregnant female mice (Marcelin et al. 2011). Pregnant mice infected with ma2009 H1N1 have greater mortality and production of pro-inflammatory cytokine and chemokines in the lungs, including TNF-α, CCL2, CCL3, and CXCL1, as compared with nonpregnant female mice (Chan et al. 2010; Marcelin et al. 2011; Uchide et al. 2012). Whether control of virus replication is diminished during pregnancy is not clear because data regarding differences in viral loads are contradictory (Chan et al. 2010; Marcelin et al. 2011).

6.5.2 Vaccination

Infection of pregnant women with either seasonal or pandemic influenza viruses can negatively affect the outcome of pregnancy by increasing the risk of spontaneous abortions, stillbirths, fetal weight loss, and fetal prematurity (Griffiths et al. 1980; Harris 1919; Hardy et al. 1961; Jamieson et al. 2009; Doyle et al. 2013; Creanga et al. 2011). Due to this, most countries recommend the use of vaccines in pregnant women.

The TIV vaccine is safe, is highly efficacious, and has no reported adverse effects on pregnancy outcomes (Tamma et al. 2009; Beau et al. 2014; Moro et al. 2012). During the 2009 H1N1 pandemic, a monovalent vaccine was made available, and there is no evidence of any risk to the mother or her fetus (Chambers et al. 2013; Louik et al. 2013). Moreover, vaccination of pregnant women induces the same amount of antibodies and similar pro-inflammatory response as in nonpregnant women (Christian et al. 2013).

6.6 Hypothesized Mechanisms Mediating Sex Differences in Response to Influenza Virus Infection and Vaccination

6.6.1 Estrogens

Estrogens, such as 17β-estradiol (E2), occur in high concentrations in nonpregnant as well as pregnant females. E2 is responsible for the majority of the "classic" estrogenic effects in reproductive and nonreproductive tissues. Estrogen receptors (ERs), including genomic and non-genomic varieties, are expressed in lymphoid tissue cells as well as in lymphocytes, macrophages, neutrophils, and dendritic cells (DCs) (Kovats et al. 2010). As detailed in Chap. 1, E2 affects several aspects of innate immunity, including the functional activity of DCs, macrophages, and neutrophils, which influence downstream adaptive immune responses. E2 has bipotential effects on monocytes and macrophages, with low doses enhancing pro-inflammatory cytokine production (e.g., IL-1, IL-6, and TNF-α) and high concentrations reducing production of these cytokines (Bouman et al. 2005). E2 has similar dose-dependent effects on cellular and humoral immune responses, with low E2 concentrations promoting Th1 responses and cell-mediated immunity but high concentrations of E2 augmenting Th2 responses and humoral immunity (Straub 2007). E2 regulates pro-inflammatory responses that are transcriptionally mediated by NF-κB through a negative feedback/transrepressive interaction with NF-κB (Kalaitzidis and Gilmore 2005; Dai et al. 2007).

Murine models of influenza A virus pathogenesis demonstrate that E2 treatment protects females against infection-induced morbidity and mortality (Robinson et al. 2011b, 2014; Nguyen et al. 2011; Pazos et al. 2012b). Treatment of these mice with E2 would appear to protect against influenza A virus infection by dampening the inflammatory responses associated with tissue damage, including excessive production of IFNγ, TNFα, and CCL2, and by promoting higher antibody responses to influenza vaccination (Robinson et al. 2011b, 2014; Nguyen et al. 2011; Pazos et al. 2012b). Some (Pazos et al. 2012b), but not all (Robinson et al. 2011b, 2014), studies suggest that treatment of females with E2 affects type I IFN responses and virus replication in the lungs. Treatment with E2 also increases production of chemoattractants for neutrophils, including CCL3 and CXCL1, pulmonary infiltration of neutrophils, and cytokine production by virus-specific CD8+ T cells as compared with placebo-treated females (Robinson et al. 2014). Neutrophils are critical regulators of inflammation, virus clearance, and tissue repair during influenza infection (Tate et al. 2009, 2012). Depletion of neutrophils in E2-treated females reverses the protective effects of E2 on the outcome of A/PR8 infection and increases inflammatory cytokine production (Robinson et al. 2014). These data indicate that neutrophils play a fundamental role in the protective effects of E2 against influenza A virus infection, at least in female mice.

6.6.2 *Progesterone*

Progesterone (P4) is produced primarily by the corpus luteum during the menstrual cycle in nonpregnant females and its production is sustained at high levels by the placenta during pregnancy (Brisken 2013). P4 signals through the progesterone receptor (PR) but also to a lesser extent through the glucocorticoid (GR) and mineralocorticoid receptor (Hapgood et al. 2013). PRs are present on many different immune cell types, including NK cells, macrophages, DCs, T cells, as well as nonimmune cells such as epithelial cells, endothelial cells, and neuronal cells (Teilmann et al. 2006; Jain et al. 2012).

In vitro studies show that P4 can alter the immune environment by promoting an anti-inflammatory milieu. It does so by modulating innate immune cells as well as skewing the cell-mediated immune response toward an anti-inflammatory, regulatory phenotype. In the presence of P4, macrophages and DCs have a lower state of activation; produce higher levels of anti-inflammatory cytokines, such as IL-10; and produce lower amounts of pro-inflammatory cytokines, such as IL-1β and TNF-α, as compared with nonhormone-treated cells (Butts et al. 2007; Jones et al. 2010; Kyurkchiev et al. 2007). When cord blood cells are treated with P4, the percentages of Foxp3+ Tregs increase, whereas the percentages of Th17 cells decrease (Lee et al. 2011). Similarly, in pregnant mice treated with P4, the proportions of Tregs in the uterus are increased (Mao et al. 2010). In humans, Tregs are also increased during pregnancy, mostly during the second and third trimester (Mjosberg et al. 2009; Saito et al. 2010).

To date, no published studies have evaluated the effects of progesterone on the outcome of influenza A virus infection. However, given the known actions of P4 described above, P4 might suppress or downregulate the inflammatory response during influenza A virus infection and potentially contribute to the worse outcome of influenza A virus during pregnancy. Alternatively, P4 may help to alleviate the pathology caused by the inflammatory response triggered during influenza A virus infection. Repair of the inflammatory damage induced in the lungs following influenza A virus infection is generally orchestrated by Tregs and cytokines, including IL-10 and TGF-β, along with remodeling by epithelial cells (Sun et al. 2009). Although the role of P4 in the repair of lung epithelium has not yet been demonstrated, P4 promotes repair of myelin fibers in the central nervous system as well as in the endometrial epithelium (Khan et al. 2005; Schumacher et al. 2012). Further, PRs are located in epithelial cells in the lungs (Jain et al. 2012). Taken together, these data suggest that P4 may play a significant role in influenza pathogenesis in both pregnant and nonpregnant females.

6.6.3 Androgens

Androgens are produced and released from Leydig cells in the testes, and occur in higher concentrations in postpubertal males than in females. Generally, androgens, including dihydrotestosterone (DHT) and testosterone (T), suppress the activity of immune cells (Roberts et al. 2001; Olsen and Kovacs 1996). Androgen receptors are expressed on several immune cell types, including monocytes, macrophages, DCs, NK cells, and lymphocytes (Lai et al. 2012). Exposure to T or DHT reduces the production of pro-inflammatory products, including TNF-α (D'Agostino et al. 1999), and increases the synthesis of anti-inflammatory cytokines, including IL-10 and TGF-β, indicating that androgens impact inflammatory responses by signaling through the androgen receptor (D'Agostino et al. 1999; Liva and Voskuhl 2001) (Gold et al. 2008). Hypogonadal men (i.e., androgen deficiency) have higher inflammatory cytokine (e.g., IL-1β, IL-2, TNF-α, and CCL3) concentrations, leptin levels, antibody titers, and CD4+:CD8+ T cell ratios than do healthy men (Kocar et al. 2000; Musabak et al. 2003; Malkin et al. 2004; Kalinchenko et al. 2010; Bobjer et al. 2013).

The anti-inflammatory effects of androgens may reflect the inhibitory effects of androgen receptor signaling mechanisms on transcriptional factors that mediate the production of pro-inflammatory and antiviral cytokines (McKay and Cidlowski 1999). Androgens also enhance the expression of peroxisome proliferator-activated receptor-α (PPAR-α) in T cells by engagement with androgen response elements in the promoter of the PPAR-α gene, which can repress the activity of NF-κB and cJun to control inflammation in males (Dunn et al. 2007). Castration of male mice reduces survival from influenza A virus infection relative to gonadally intact controls suggesting that androgens may be protective against influenza A virus infection (Robinson et al. 2011a, b). If men are stratified based on whether their circulating testosterone concentrations are above (high) or below (low) the median, those men with high testosterone concentrations have the lowest antibody responses following TIV administration when compared with women, which appears to be caused by androgen regulation of lipid biosynthesis (Furman et al. 2013). Additional studies must determine the mechanisms mediating how androgens alter immune responses to influenza virus infection and vaccination in males.

6.6.4 Genetic Mechanisms

Although direct effects of sex steroids may cause sex differences in physiology, another hypothesis is that genes on the X chromosome, the Y chromosome, or both alter the expression of sexually dimorphic phenotypes directly in non-gonadal tissues through mechanisms other than gonadal hormones (Lenz 1931; Purtilo and Sullivan 1979; Arnold and Chen 2009). Many genes on the X chromosome regulate immune function and play an important role in modulating sex differences

in the development of immune-related diseases (Libert et al. 2010). These immune-related genes code for proteins ranging from pathogen recognition receptors (PRRs) (e.g., *Tlr7* and *Tlr8*) to cytokine receptors (e.g., *Il2rg* and *Il13ra2*) and transcriptional factors (e.g., *Foxp3*) (Fish 2008). There is higher expression levels of *Tlr7* in females than males (Pisitkun et al. 2006) and DCs isolated from women produce twice as much IFN-α in response to TLR7 ligands than do DCs from men (Meier et al. 2009). Polymorphisms in Y chromosome genes also affect sex-dependent susceptibility to viral infection (Case et al. 2012). The expression of X-linked genes may also be affected by X-linked miRNAs. MiRNAs are small noncoding RNAs that regulate genes expression at a posttranscriptional level and play a critical role in maintaining immunological homeostasis. Dysregulation of miRNA expression may underlie development of immune-mediated diseases, ranging from cancers to autoimmune diseases (Pinheiro et al. 2011). There are a disproportionately higher number of miRNAs located on the X chromosome than on any autosomal chromosome, which is hypothesized to contribute to sex-specific development of immune-mediated diseases (Pinheiro et al. 2011). Interpretation of sex differences in the expression of X-linked genes, however, is challenging because sex hormones or sex chromosome complement can still contribute to the observed differential gene expression (Arnold and Lusis 2012).

The *Sry* gene on the Y chromosome causes testes formation and testosterone synthesis leading to male-typic development of many phenotypes, whereas the absence of *Sry* results in ovaries and female-typic development (Koopman et al. 1991). The "four core genotypes" (FCG) mouse model has been developed to investigate the impact of sex chromosomes (XX vs. XY) and gonadal type (testes vs. ovaries) on phenotypes. In FCG mice, *Sry* is deleted from the Y chromosome and an *Sry* transgene is inserted onto an autosome. Deletion of the *Sry* gene results in XYminus (XY^-) mice that are gonadal females (i.e., with ovaries), whereas insertion of the *Sry* transgene onto an autosome in XX or XY^- mice (XX*Sry* and XY^-*Sry*) results in gonadal males (i.e., with testes). Depletion of gonadal steroids by gonadectomy of FCG mice unmasks effects of sex chromosome complement on behavior, brain function, renal function, and susceptibility to autoimmune disease (Arnold and Chen 2009; Smith-Bouvier et al. 2008). We examined whether sex chromosome complement affects susceptibility to influenza A virus infection and found that sex chromosome complement did not affect influenza pathogenesis (Robinson et al. 2011a). Among those FCG animals that died following inoculation with A/PR8, the average day of death was later for gonadal male than gonadal female mice, regardless of whether their sex chromosome complement was XX or XY. These data support the hypothesis that sex differences in influenza virus pathogenesis are predominately mediated by sex steroid hormones rather than by sex chromosome complement.

6.7 Conclusions and Future Directions

Males and females are biologically different. There are significant gaps in our understanding of the precise mechanisms mediating sex-biased immune responses and how this affects the outcome of influenza infection and vaccination. Future research must continue to define the pathways mediating how hormones, genes, and genetic polymorphisms alter the functioning of immune cells to change the pathogenesis of influenza as well as the outcome of vaccination. Future studies should continue to consider the age and reproductive status of females as well as whether females are using exogenous hormones (either through contraceptives or replacement therapy) at the time of infection, drug treatment, or both. The observation that sex differences in the outcome of infection and in responses to antiviral treatments for hepatitis depend on the hormonal status, and not merely the age, of women (Villa et al. 2011) is an important observation that should stimulate similar studies for other diseases, including influenza. Additionally, whether the hormonal milieu at the time of vaccination influences immune responses and long-term protection against influenza should be examined.

The recommendation of funding agencies, universities, and journals should be that clinicians, epidemiologists, and basic biomedical scientists design experiments that include both males and females, develop a priori hypotheses that the sexes will differ in their responses to and the outcome of infection and treatments, and statistically analyze outcome data by sex. The end goal should be that clinicians and researchers alike consider the sex of their patients or animals when designing and administering treatments for viral diseases because the outcomes will likely differ. Consideration of biological sex when formulating and administering therapies or prophylaxis treatments for viral diseases, including influenza, may improve the efficacy and long-term protection in both males and females.

References

Ailes EC, Newsome K, Williams JL, McIntyre AF, Jamieson DJ, Finelli L, Honein MA (2013) CDC pregnancy flu line: monitoring severe illness among pregnant women with influenza. Matern Child Health J. doi:10.1007/s10995-013-1415-6

Arima Y, Vong S (2013) Human infections with avian influenza A(H7N9) virus in China: preliminary assessments of the age and sex distribution. Western Pac Surveill Response J 4 (2):1–3. doi:10.5365/wpsar.2013.4.2.005

Arnold AP, Chen X (2009) What does the "four core genotypes" mouse model tell us about sex differences in the brain and other tissues? Front Neuroendocrinol 30(1):1–9. doi:10.1016/j.yfrne.2008.11.001

Arnold AP, Lusis AJ (2012) Understanding the sexome: measuring and reporting sex differences in gene systems. Endocrinology 153(6):2551–2555

Bean-Mayberry B, Yano EM, Mor MK, Bayliss NK, Xu X, Fine MJ (2009) Does sex influence immunization status for influenza and pneumonia in older veterans? J Am Geriatr Soc 57 (8):1427–1432. doi:10.1111/j.1532-5415.2009.02316.x

Beau AB, Hurault-Delarue C, Vidal S, Guitard C, Vayssiere C, Petiot D, Montastruc JL, Damase-Michel C, Lacroix I (2014) Pandemic A/H1N1 influenza vaccination during pregnancy: a comparative study using the EFEMERIS database. Vaccine 32(11):1254–1258. doi:10.1016/j.vaccine.2014.01.021

Beigi RH (2012) Influenza during pregnancy: a cause of serious infection in obstetrics. Clin Obstet Gynecol 55(4):914–926. doi:10.1097/GRF.0b013e31827146bd

Blanchon T, Mentre F, Charlois-Ou C, Dornic Q, Mosnier A, Bouscambert M, Carrat F, Duval X, Enouf V, Leport C (2011) Factors associated with clinical and virological response in patients treated with oseltamivir or zanamivir for influenza A during the 2008–2009 winter. Clin Microbiol Infect. doi:10.1111/j.1469-0691.2011.03751.x

Bobjer J, Katrinaki M, Tsatsanis C, Lundberg Giwercman Y, Giwercman A (2013) Negative association between testosterone concentration and inflammatory markers in young men: a nested cross-sectional study. PLoS ONE 8(4):e61466. doi:10.1371/journal.pone.0061466

Bouman A, Heineman MJ, Faas MM (2005) Sex hormones and the immune response in humans. Hum Reprod Update 11(4):411–423

Bouvier NM, Lowen AC (2010) Animal Models for Influenza Virus Pathogenesis and Transmission. Viruses 2(8):1530–1563. doi:10.3390/v20801530

Briand S, Fukuda K (2009) Avian influenza A (H5N1) virus and 2 fundamental questions. J Infect Dis 199(12):1717–1719. doi:10.1086/599209

Brisken C (2013) Progesterone signalling in breast cancer: a neglected hormone coming into the limelight. Nat Rev Cancer 13(6):385–396. doi:10.1038/nrc3518

Butts CL, Shukair SA, Duncan KM, Bowers E, Horn C, Belyavskaya E, Tonelli L, Sternberg EM (2007) Progesterone inhibits mature rat dendritic cells in a receptor-mediated fashion. Int Immunol 19(3):287–296

Case LK, Toussaint L, Moussawi M, Roberts B, Saligrama N, Brossay L, Huber SA, Teuscher C (2012) Chromosome y regulates survival following murine coxsackievirus b3 infection. G3 (Bethesda) 2(1):115–121. doi:10.1534/g3.111.001610

Cate TR, Couch RB, Parker D, Baxter B (1983) Reactogenicity, immunogenicity, and antibody persistence in adults given inactivated influenza virus vaccines - 1978. Rev Infect Dis 5 (4):737–747

CDC (2014a) Avian Influenza A (H7N9) Virus. Centers for Disease Control and Prevention. http://www.cdc.gov/flu/avianflu/h7n9-virus.htm

CDC (2014b) Highly Pathogenic Avian Influenza A (H5N1) Virus. Centers for Disease Control Prevention. http://www.cdc.gov/flu/avianflu/h5n1-virus.htm

CDC (2014c) Laboratory-confirmed influenza hospitalizations, 2009–2010. Centers for Disease Control and Prevention. http://gis.cdc.gov/grasp/fluview/FluHospChars.html

CDC (2014d) Laboratory-Confirmed Influenza Hospitalizations, 2013–2014. Centers for Diseases Control and Prevention. http://gis.cdc.gov/grasp/fluview/FluHospChars.html

Chambers CD, Johnson D, Xu R, Luo Y, Louik C, Mitchell AA, Schatz M, Jones KL, Group OCR (2013) Risks and safety of pandemic h1n1 influenza vaccine in pregnancy: birth defects, spontaneous abortion, preterm delivery, and small for gestational age infants. Vaccine 31 (44):5026–5032. doi:10.1016/j.vaccine.2013.08.097

Chan KH, Zhang AJ, To KK, Chan CC, Poon VK, Guo K, Ng F, Zhang QW, Leung VH, Cheung AN, Lau CC, Woo PC, Tse H, Wu W, Chen H, Zheng BJ, Yuen KY (2010) Wild type and mutant 2009 pandemic influenza A (H1N1) viruses cause more severe disease and higher mortality in pregnant BALB/c mice. PLoS ONE 5(10):e13757. doi:10.1371/journal.pone.0013757

Chor JS, Ngai KL, Goggins WB, Wong MC, Wong SY, Lee N, Leung TF, Rainer TH, Griffiths S, Chan PK (2009) Willingness of Hong Kong healthcare workers to accept pre-pandemic influenza vaccination at different WHO alert levels: two questionnaire surveys. Br Med J 339:b3391

Christian LM, Porter K, Karlsson E, Schultz-Cherry S, Iams JD (2013) Serum proinflammatory cytokine responses to influenza virus vaccine among women during pregnancy versus non-pregnancy. Am J Reprod Immunol 70(1):45–53. doi:10.1111/aji.12117

Chudasama RK, Patel UV, Verma PB (2013) Characteristics of Hospitalized Patients with Severe and Non-Severe Pandemic Influenza A (H1N1) in Saurashtra Region, India (Two Waves Analysis). J Family Med Prim Care 2(2):182–187. doi:10.4103/2249-4863.117397

Cook IF (2009) Sex differences in injection site reactions with human vaccines. Hum Vaccin 5 (7):441–449

Cowling BJ, Jin L, Lau EH, Liao Q, Wu P, Jiang H, Tsang TK, Zheng J, Fang VJ, Chang Z, Ni MY, Zhang Q, Ip DK, Yu J, Li Y, Wang L, Tu W, Meng L, Wu JT, Luo H, Li Q, Shu Y, Li Z, Feng Z, Yang W, Wang Y, Leung GM, Yu H (2013) Comparative epidemiology of human infections with avian influenza A H7N9 and H5N1 viruses in China: a population-based study of laboratory-confirmed cases. Lancet 382(9887):129–137. doi:10.1016/s0140-6736(13)61171-x

Cox S, Posner SF, McPheeters M, Jamieson DJ, Kourtis AP, Meikle S (2006) Hospitalizations with respiratory illness among pregnant women during influenza season. Obstet Gynecol 107 (6):1315–1322

Creanga AA, Kamimoto L, Newsome K, D'Mello T, Jamieson DJ, Zotti ME, Arnold KE, Baumbach J, Bennett NM, Farley MM, Gershman K, Kirschke D, Lynfield R, Meek J, Morin C, Reingold A, Ryan P, Schaffner W, Thomas A, Zansky S, Finelli L, Honein MA (2011) Seasonal and 2009 pandemic influenza A (H1N1) virus infection during pregnancy: a population-based study of hospitalized cases. Am J Obstet Gynecol 204(6 Suppl 1):S38–S45

Crighton EJ, Moineddin R, Mamdani M, Upshur RE (2004) Influenza and pneumonia hospitalizations in Ontario: a time-series analysis. Epidemiol Infect 132(6):1167–1174

Crighton EJ, Elliott SJ, Kanaroglou P, Moineddin R, Upshur RE (2008) Spatio-temporal analysis of pneumonia and influenza hospitalizations in Ontario, Canada. Geospatial Health 2 (2):191–202

D'Agostino P, Milano S, Barbera C, Di Bella G, La Rosa M, Ferlazzo V, Farruggio R, Miceli DM, Miele M, Castagnetta L, Cillari E (1999) Sex hormones modulate inflammatory mediators produced by macrophages. Ann N Y Acad Sci 876:426–429

Dai R, Phillips RA, Ahmed SA (2007) Despite inhibition of nuclear localization of NF-kappa B p65, c-Rel, and RelB, 17-beta estradiol up-regulates NF-kappa B signaling in mouse splenocytes: the potential role of Bcl-3. J Immunol 179(3):1776–1783

De Clercq E (2006) Antiviral agents active against influenza A viruses. Nat Rev Drug Discov 5 (12):1015–1025

de Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN, Hoang DM, Chau NV, Khanh TH, Dong VC, Qui PT, Cam BV, Hado Q, Guan Y, Peiris JS, Chinh NT, Hien TT, Farrar J (2006) Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. Nat Med 12(10):1203–1207. doi:10.1038/nm1477

Doyle TJ, Goodin K, Hamilton JJ (2013) Maternal and neonatal outcomes among pregnant women with 2009 pandemic influenza A(H1N1) illness in Florida, 2009–2010: a population-based cohort study. PLoS ONE 8(10):e79040. doi:10.1371/journal.pone.0079040

Dudley JP (2009) Age-specific infection and death rates for human A(H5N1) avian influenza in Egypt. Euro Surveill 14(18)

Dudley JP, Mackay IM (2013) Age-specific and sex-specific morbidity and mortality from avian influenza A(H7N9). J Clin Virol 58(3):568–570. doi:10.1016/j.jcv.2013.09.004

Dunn SE, Ousman SS, Sobel RA, Zuniga L, Baranzini SE, Youssef S, Crowell A, Loh J, Oksenberg J, Steinman L (2007) Peroxisome proliferator-activated receptor (PPAR)alpha expression in T cells mediates gender differences in development of T cell-mediated autoimmunity. J Exp Med 204(2):321–330

Elenkov IJ, Wilder RL, Bakalov VK, Link AA, Dimitrov MA, Fisher S, Crane M, Kanik KS, Chrousos GP (2001) IL-12, TNF-alpha, and hormonal changes during late pregnancy and early

postpartum: implications for autoimmune disease activity during these times. J Clin Endocrinol Metab 86(10):4933–4938. doi:10.1210/jcem.86.10.7905

Endrich MM, Blank PR, Szucs TD (2009) Influenza vaccination uptake and socioeconomic determinants in 11 European countries. Vaccine 27(30):4018–4024

Engler RJ, Nelson MR, Klote MM, VanRaden MJ, Huang CY, Cox NJ, Klimov A, Keitel WA, Nichol KL, Carr WW, Treanor JJ (2008) Half- vs full-dose trivalent inactivated influenza vaccine (2004–2005): age, dose, and sex effects on immune responses. Arch Intern Med 168 (22):2405–2414. doi:10.1001/archinternmed.2008.513

Eshima N, Tokumaru O, Hara S, Bacal K, Korematsu S, Tabata M, Karukaya S, Yasui Y, Okabe N, Matsuishi T (2011) Sex- and age-related differences in morbidity rates of 2009 pandemic influenza A H1N1 virus of swine origin in Japan. PLoS ONE 6(4):e19409. doi:10.1371/journal.pone.0019409 PONE-D-10-04813 [pii]

Falsey AR, Treanor JJ, Tornieporth N, Capellan J, Gorse GJ (2009) Randomized, double-blind controlled phase 3 trial comparing the immunogenicity of high-dose and standard-dose influenza vaccine in adults 65 years of age and older. J Infect Dis 200(2):172–180. doi:10.1086/599790

Fasina FO, Ifende VI, Ajibade AA (2010) Avian influenza A(H5N1) in humans: lessons from Egypt. Euro Surveill 15(4):19473

Fish EN (2008) The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol 8(9):737–744

Fofie AE, Fewell JE, Moore SL (2005) Pregnancy influences the plasma cytokine response to intraperitoneal administration of bacterial endotoxin in rats. Exp Physiol 90(1):95–101. doi:10.1113/expphysiol.2004.028613

Furman D, Hejblum BP, Simon N, Jojic V, Dekker CL, Thiebaut R, Tibshirani RJ, Davis MM (2013) Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. Proc Natl Acad Sci U S A. doi:10.1073/pnas.1321060111

Gagnon A, Miller MS, Hallman SA, Bourbeau R, Herring DA, Earn DJD, Madrenas J (2013) Age-specific mortality during the 1918 influenza pandemic: unravelling the mystery of high young adult mortality. PLoS ONE 8(8):e69586. doi:10.1371/journal.pone.0069586

Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, Chen J, Jie Z, Qiu H, Xu K, Xu X, Lu H, Zhu W, Gao Z, Xiang N, Shen Y, He Z, Gu Y, Zhang Z, Yang Y, Zhao X, Zhou L, Li X, Zou S, Zhang Y, Li X, Yang L, Guo J, Dong J, Li Q, Dong L, Zhu Y, Bai T, Wang S, Hao P, Yang W, Zhang Y, Han J, Yu H, Li D, Gao GF, Wu G, Wang Y, Yuan Z, Shu Y (2013) Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med 368(20):1888–1897. doi:10.1056/NEJMoa1304459

Gold SM, Chalifoux S, Giesser BS, Voskuhl RR (2008) Immune modulation and increased neurotrophic factor production in multiple sclerosis patients treated with testosterone. J Neuroinflammation 5:32. doi:10.1186/1742-2094-5-32

Griffioen M, Halsey N (2014) Gender differences in immediate hypersensitivity reactions to vaccines: a review of the literature. Public Health Nurs 31(3):206–214. doi:10.1111/phn.12073

Griffiths PD, Ronalds CJ, Heath RB (1980) A prospective study of influenza infections during pregnancy. J Epidemiol Community Health 34(2):124–128

Guan Y, Poon LL, Cheung CY, Ellis TM, Lim W, Lipatov AS, Chan KH, Sturm-Ramirez KM, Cheung CL, Leung YH, Yuen KY, Webster RG, Peiris JS (2004) H5N1 influenza: a protean pandemic threat. Proc Natl Acad Sci U S A 101(21):8156–8161

Hapgood JP, Africander D, Louw R, Ray RM, Rohwer JM (2013) Potency of progestogens used in hormonal therapy: toward understanding differential actions. J Steroid Biochem Mol Biol. doi:10.1016/j.jsbmb.2013.08.001

Hardy JM, Azarowicz EN, Mannini A, Medearis DN Jr, Cooke RE (1961) The effect of Asian influenza on the outcome of pregnancy, Baltimore, 1957–1958. Am J Public Health Nation Health 51:1182–1188

Harris JW (1919) Influenza occurring in pregnant women: a statistical study of thirteen hundred and fifty cases. J Am Med Assoc 72(14):978–980. doi:10.1001/jama.1919.02610140008002

Hien ND, Ha NH, Van NT, Ha NT, Lien TT, Thai NQ, Trang VD, Shimbo T, Takahashi Y, Kato Y, Kawana A, Akita S, Kudo K (2009) Human infection with highly pathogenic avian influenza virus (H5N1) in northern Vietnam, 2004–2005. Emerg Infect Dis 15(1):19–23

Jacobs JH, Archer BN, Baker MG, Cowling BJ, Heffernan RT, Mercer G, Uez O, Hanshaoworakul W, Viboud C, Schwartz J, Tchetgen Tchetgen E, Lipsitch M (2012) Searching for sharp drops in the incidence of pandemic A/H1N1 influenza by single year of age. PLoS ONE 7(8):e42328. doi:10.1371/journal.pone.0042328

Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J, Sugerman DE, Druckenmiller JK, Ritger KA, Chugh R, Jasuja S, Deutscher M, Chen S, Walker JD, Duchin JS, Lett S, Soliva S, Wells EV, Swerdlow D, Uyeki TM, Fiore AE, Olsen SJ, Fry AM, Bridges CB, Finelli L, Pandemic Influenza AVHIT (2009) Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. N Engl J Med 361(20):1935–1944. doi:10.1056/NEJMoa0906695

Jain R, Ray JM, Pan JH, Brody SL (2012) Sex hormone-dependent regulation of cilia beat frequency in airway epithelium. Am J Respir Cell Mol Biol 46(4):446–453. doi:10.1165/rcmb.2011-0107OC

Jamieson DJ, Honein MA, Rasmussen SA, Williams JL, Swerdlow DL, Biggerstaff MS, Lindstrom S, Louie JK, Christ CM, Bohm SR, Fonseca VP, Ritger KA, Kuhles DJ, Eggers P, Bruce H, Davidson HA, Lutterloh E, Harris ML, Burke C, Cocoros N, Finelli L, MacFarlane KF, Shu B, Olsen SJ (2009) H1N1 2009 influenza virus infection during pregnancy in the USA. Lancet 374(9688):451–458. doi:10.1016/s0140-6736(09)61304-0

Jensen-Fangel S, Mohey R, Johnsen SP, Andersen PL, Sorensen HT, Ostergaard L (2004) Gender differences in hospitalization rates for respiratory tract infections in Danish youth. Scand J Infect Dis 36(1):31–36

Jimenez-Garcia R, Hernandez-Barrera V, de Andres AL, Jimenez-Trujillo I, Esteban-Hernandez J, Carrasco-Garrido P (2010) Gender influence in influenza vaccine uptake in Spain: time trends analysis (1995–2006). Vaccine 28(38):6169–6175. doi:10.1016/j.vaccine.2010.07.029

Jones LA, Kreem S, Shweash M, Paul A, Alexander J, Roberts CW (2010) Differential modulation of TLR3- and TLR4-mediated dendritic cell maturation and function by progesterone. J Immunol 185(8):4525–4534. doi:10.4049/jimmunol.0901155

Kalaitzidis D, Gilmore TD (2005) Transcription factor cross-talk: the estrogen receptor and NF-kappaB. Trends Endocrinol Metabol 16(2):46–52. doi:10.1016/j.tem.2005.01.004

Kalinchenko SY, Tishova YA, Mskhalaya GJ, Gooren LJ, Giltay EJ, Saad F (2010) Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. Clin Endocrinol (Oxf) 73(5):602–612. doi:10.1111/j.1365-2265.2010.03845.x

Kayali G, Webby RJ, Ducatez MF, El Shesheny RA, Kandeil AM, Govorkova EA, Mostafa A, Ali MA (2011) The epidemiological and molecular aspects of influenza H5N1 viruses at the human-animal interface in Egypt. PLoS ONE 6(3):e17730. doi:10.1371/journal.pone.0017730

Khan KN, Masuzaki H, Fujishita A, Kitajima M, Sekine I, Matsuyama T, Ishimaru T (2005) Estrogen and progesterone receptor expression in macrophages and regulation of hepatocyte growth factor by ovarian steroids in women with endometriosis. Hum Reprod 20(7):2004–2013. doi:10.1093/humrep/deh897

Khurana S, Verma N, Talaat KR, Karron RA, Golding H (2012) Immune response following H1N1pdm09 vaccination: differences in antibody repertoire and avidity in young adults and elderly populations stratified by age and gender. J Infect Dis 205(4):610–620. doi:10.1093/infdis/jir791

Kilbourne ED (2006) Influenza pandemics of the 20th century. Emerg Infect Dis 12(1):9–14. doi:10.3201/eid1201.051254

Klein SL (2012) Sex influences immune responses to viruses, and efficacy of prophylaxis and treatments for viral diseases. BioEssays 34(12):1050–1059. doi:10.1002/bies.201200099

Klein SL, Jedlicka A, Pekosz A (2010a) The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis 10(5):338–349. doi:10.1016/S1473-3099(10)70049-9

Klein SL, Passaretti C, Anker M, Olukoya P, Pekosz A (2010b) The impact of sex, gender and pregnancy on 2009 H1N1 disease. Biol Sex Differ 1(1):5. doi:10.1186/2042-6410-1-5

Kocar IH, Yesilova Z, Ozata M, Turan M, Sengul A, Ozdemir I (2000) The effect of testosterone replacement treatment on immunological features of patients with Klinefelter's syndrome. Clin Exp Immunol 121(3):448–452

Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R (1991) Male development of chromosomally female mice transgenic for Sry. Nature 351(6322):117–121. doi:10.1038/351117a0

Kovats S, Carreras E, Agrawal H (2010) Sex steroid receptors in immune cells. In: Klein SL, Roberts CW (eds) Sex hormones and immunity to infection. Springer, Berlin, pp 53–92

Kraus TA, Sperling RS, Engel SM, Lo Y, Kellerman L, Singh T, Loubeau M, Ge Y, Garrido JL, Rodriguez-Garcia M, Moran TM (2010) Peripheral blood cytokine profiling during pregnancy and post-partum periods. Am J Reprod Immunol 64(6):411–426. doi:10.1111/j.1600-0897.2010.00889.x

Kumar A, Zarychanski R, Pinto R, Cook DJ, Marshall J, Lacroix J, Stelfox T, Bagshaw S, Choong K, Lamontagne F, Turgeon AF, Lapinsky S, Ahern SP, Smith O, Siddiqui F, Jouvet P, Khwaja K, McIntyre L, Menon K, Hutchison J, Hornstein D, Joffe A, Lauzier F, Singh J, Karachi T, Wiebe K, Olafson K, Ramsey C, Sharma S, Dodek P, Meade M, Hall R, Fowler RA (2009) Critically ill patients with 2009 influenza A(H1N1) infection in Canada. JAMA 302(17):1872–1879

Kyurkchiev D, Ivanova-Todorova E, Hayrabedyan S, Altankova I, Kyurkchiev S (2007) Female sex steroid hormones modify some regulatory properties of monocyte-derived dendritic cells. Am J Reprod Immunol 58(5):425–433. doi:10.1111/j.1600-0897.2007.00526.x

Lai JJ, Lai KP, Zeng W, Chuang KH, Altuwaijri S, Chang C (2012) Androgen receptor influences on body defense system via modulation of innate and adaptive immune systems: lessons from conditional AR knockout mice. Am J Pathol 181(5):1504–1512. doi:10.1016/j.ajpath.2012.07.008

Larcombe AN, Foong RE, Bozanich EM, Berry LJ, Garratt LW, Gualano RC, Jones JE, Dousha LF, Zosky GR, Sly PD (2011) Sexual dimorphism in lung function responses to acute influenza A infection. Influenza Other Respir Viruses. doi:10.1111/j.1750-2659.2011.00236.x

Lee JH, Ulrich B, Cho J, Park J, Kim CH (2011) Progesterone promotes differentiation of human cord blood fetal T cells into T regulatory cells but suppresses their differentiation into Th17 cells. J Immunol 187(4):1778–1787. doi:10.4049/jimmunol.1003919

Lenz F (1931) Morbidic hereditary factors. Macmillan, New York

Li Q, Zhou L, Zhou M, Chen Z, Li F, Wu H, Xiang N, Chen E, Tang F, Wang D, Meng L, Hong Z, Tu W, Cao Y, Li L, Ding F, Liu B, Wang M, Xie R, Gao R, Li X, Bai T, Zou S, He J, Hu J, Xu Y, Chai C, Wang S, Gao Y, Jin L, Zhang Y, Luo H, Yu H, He J, Li Q, Wang X, Gao L, Pang X, Liu G, Yan Y, Yuan H, Shu Y, Yang W, Wang Y, Wu F, Uyeki TM, Feng Z (2014) Epidemiology of human infections with avian influenza A(H7N9) virus in China. N Engl J Med 370(6):520–532. doi:10.1056/NEJMoa1304617

Libert C, Dejager L, Pinheiro I (2010) The X chromosome in immune functions: when a chromosome makes the difference. Nat Rev Immunol 10(8):594–604

Liem NT, Tung CV, Hien ND, Hien TT, Chau NQ, Long HT, Hien NT, le Mai Q, Taylor WR, Wertheim H, Farrar J, Khang DD, Horby P (2009) Clinical features of human influenza A (H5N1) infection in Vietnam: 2004–2006. Clin Infect Dis 48(12):1639–1646. doi:10.1086/599031

Liu SL, Wang J, Yang XH, Chen J, Huang RJ, Ruan B, He HX, Wang CM, Zhang HM, Sun Z, Xie L, Zhuang H (2013) Pandemic influenza A(H1N1) 2009 virus in pregnancy. Rev Med Virol 23(1):3–14. doi:10.1002/rmv.1712

Liva SM, Voskuhl RR (2001) Testosterone acts directly on CD4+ T lymphocytes to increase IL-10 production. J Immunol 167(4):2060–2067

Lorenzo ME, Hodgson A, Robinson DP, Kaplan JB, Pekosz A, Klein SL (2011) Antibody responses and cross protection against lethal influenza A viruses differ between the sexes in C57BL/6 mice. Vaccine 29(49):9246–9255. doi:10.1016/j.vaccine.2011.09.110

Louie JK, Acosta M, Winter K, Jean C, Gavali S, Schechter R, Vugia D, Harriman K, Matyas B, Glaser CA, Samuel MC, Rosenberg J, Talarico J, Hatch D, California Pandemic (H1N1) Working Group (2009) Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. JAMA 302(17):1896–1902. doi:10.1001/jama.2009.1583

Louie JK, Acosta M, Jamieson DJ, Honein MA, California Pandemic Working G (2010) Severe 2009 H1N1 influenza in pregnant and postpartum women in California. N Engl J Med 362 (1):27–35. doi:10.1056/NEJMoa0910444

Louik C, Ahrens K, Kerr S, Pyo J, Chambers C, Jones KL, Schatz M, Mitchell AA (2013) Risks and safety of pandemic H1N1 influenza vaccine in pregnancy: exposure prevalence, preterm delivery, and specific birth defects. Vaccine 31(44):5033–5040. doi:10.1016/j.vaccine.2013.08.096

Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH (2004) The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. J Clin Endocrinol Metab 89(7):3313–3318. doi:10.1210/jc.2003-031069 89/7/3313 [pii]

Maltezou HC, Drakoulis N, Siahanidou T, Karalis V, Zervaki E, Dotsikas Y, Loukas YL, Theodoridou M (2011) Safety and Pharmacokinetics of Oseltamivir for Prophylaxis of Neonates Exposed to Influenza H1N1. Pediatr Infect Dis J. doi:10.1097/INF.0b013e3182472f28

Mao G, Wang J, Kang Y, Tai P, Wen J, Zou Q, Li G, Ouyang H, Xia G, Wang B (2010) Progesterone increases systemic and local uterine proportions of CD4+CD25+ Treg cells during midterm pregnancy in mice. Endocrinology 151(11):5477–5488. doi:10.1210/en.2010-0426

Marcelin G, Aldridge JR, Duan S, Ghoneim HE, Rehg J, Marjuki H, Boon AC, McCullers JA, Webby RJ (2011) Fatal outcome of pandemic H1N1 2009 influenza virus infection is associated with immunopathology and impaired lung repair, not enhanced viral burden, in pregnant mice. J Virol 85(21):11208–11219. doi:10.1128/JVI. 00654-11

Markle JG, Fish EN (2014) SeXX matters in immunity. Trends Immunol 35(3):97–104. doi:10.1016/j.it.2013.10.006

Marzi M, Vigano A, Trabattoni D, Villa ML, Salvaggio A, Clerici E, Clerici M (1996) Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. Clin Exp Immunol 106(1):127–133

McKay LI, Cidlowski JA (1999) Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. Endocr Rev 20 (4):435–459

Medina RA, Garcia-Sastre A (2011) Influenza A viruses: new research developments. Nat Rev Microbiol 9(8):590–603. doi:10.1038/nrmicro2613

Meier A, Chang JJ, Chan ES, Pollard RB, Sidhu HK, Kulkarni S, Wen TF, Lindsay RJ, Orellana L, Mildvan D, Bazner S, Streeck H, Alter G, Lifson JD, Carrington M, Bosch RJ, Robbins GK, Altfeld M (2009) Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. Nat Med 15(8):955–959. doi:10.1038/nm.2004

Memoli MJ, Harvey H, Morens DM, Taubenberger JK (2013) Influenza in pregnancy. Influenza Other Respir Viruses 7(6):1033–1039. doi:10.1111/irv.12055

Merrill RM, Beard JD (2009) Influenza vaccination in the United States, 2005–2007. Med Sci Monit 15(7):PH92–PH100

Mjosberg J, Svensson J, Johansson E, Hellstrom L, Casas R, Jenmalm MC, Boij R, Matthiesen L, Jonsson JI, Berg G, Ernerudh J (2009) Systemic reduction of functionally suppressive CD4dimCD25highFoxp3+ Tregs in human second trimester pregnancy is induced by progesterone and 17{beta}-estradiol. J Immunol 183(1):759–769

Moorman JE, Rudd RA (2007) National surveillance for asthma- United States, 1980–2004. MMWR Surveillance summaries: morbidity and mortality weekly report, vol 56. CDC

Morens DM, Taubenberger JK, Fauci AS (2008) Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. J Infect Dis 198(7):962–970. doi:10.1086/591708

Moro PL, Tepper NK, Grohskopf LA, Vellozzi C, Broder K (2012) Safety of seasonal influenza and influenza A (H1N1) 2009 monovalent vaccines in pregnancy. Expert Rev Vaccines 11 (8):911–921. doi:10.1586/erv.12.72

Mosby LG, Rasmussen SA, Jamieson DJ (2011) 2009 pandemic influenza A (H1N1) in pregnancy: a systematic review of the literature. Am J Obstet Gynecol 205(1):10–18. doi:10.1016/j.ajog.2010.12.033

Musabak U, Bolu E, Ozata M, Oktenli C, Sengul A, Inal A, Yesilova Z, Kilciler G, Ozdemir IC, Kocar IH (2003) Gonadotropin treatment restores in vitro interleukin-1beta and tumour necrosis factor-alpha production by stimulated peripheral blood mononuclear cells from patients with idiopathic hypogonadotropic hypogonadism. Clin Exp Immunol 132(2):265–270

Neuzil KM, Reed GW, Mitchel EF, Simonsen L, Griffin MR (1998) Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. Am J Epidemiol 148(11):1094–1102

Ng WF, To KF (2007) Pathology of human H5N1 infection: new findings. Lancet 370 (9593):1106–1108. doi:10.1016/s0140-6736(07)61490-1

Nguyen DC, Masseoud F, Lu X, Scinicariello F, Sambhara S, Attanasio R (2011) 17beta-Estradiol restores antibody responses to an influenza vaccine in a postmenopausal mouse model. Vaccine 29(14):2515–2518. doi:10.1016/j.vaccine.2011.01.080

Nichol KL, Margolis KL, Lind A, Murdoch M, McFadden R, Hauge M, Magnan S, Drake M (1996) Side effects associated with influenza vaccination in healthy working adults. A randomized, placebo-controlled trial. Arch Intern Med 156(14):1546–1550

Nogueira PJ, Nunes B, Machado A, Rodrigues E, Gomez V, Sousa L, Falcao JM (2009) Early estimates of the excess mortality associated with the 2008–9 influenza season in Portugal. Euro Surveill 14(18)

Noymer A, Garenne M (2000) The 1918 influenza epidemic's effects on sex differentials in mortality in the United States. Popul Dev Rev 26(3):565–581

Oliveira W, Carmo E, Penna G, Kuchenbecker R, Santos H, Araujo W, Malaguti R, Duncan B, Schmidt M (2009) Pandemic H1N1 influenza in Brazil: analysis of the first 34,506 notified cases of influenza-like illness with severe acute respiratory infection (SARI). Euro surveillance : bulletin Europeen sur les maladies transmissibles. Eur Commun Dis Bull 14(42)

Olsen NJ, Kovacs WJ (1996) Gonadal steroids and immunity. Endocr Rev 17(4):369–384

Ontario (2009) Ontario novel H1N1 influenza A virus epidemiological summary. Ministry of Health

Opstelten W, van Essen GA, Ballieux MJ, Goudswaard AN (2008) Influenza immunization of Dutch general practitioners: vaccination rate and attitudes towards vaccination. Vaccine 26 (47):5918–5921. doi:10.1016/j.vaccine.2008.08.049

Pazos M, Sperling RS, Moran TM, Kraus TA (2012a) The influence of pregnancy on systemic immunity. Immunol Res 54(1–3):254–261. doi:10.1007/s12026-012-8303-9

Pazos MA, Kraus TA, Munoz-Fontela C, Moran TM (2012b) Estrogen mediates innate and adaptive immune alterations to influenza infection in pregnant mice. PLoS ONE 7(7): e40502. doi:10.1371/journal.pone.0040502 PONE-D-11-22626 [pii]

Pinheiro I, Dejager L, Libert C (2011) X-chromosome-located microRNAs in immunity: might they explain male/female differences? The X chromosome-genomic context may affect X-located miRNAs and downstream signaling, thereby contributing to the enhanced immune response of females. BioEssays. doi:10.1002/bies.201100047

Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, Bolland S (2006) Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science 312(5780):1669–1672

Pittman PR (2002) Aluminum-containing vaccine associated adverse events: role of route of administration and gender. Vaccine 20(Suppl 3):S48–S50

Purtilo DT, Sullivan JL (1979) Immunological bases for superior survival of females. Am J Dis Child 133(12):1251–1253

Qi X, Cui L, Xu K, Wu B, Tang F, Bao C, Zhu Y, Zhou MH, Wang H (2014) Avian influenza A (H7N9) virus infection in pregnant woman, China, 2013. Emerg Infect Dis 20(2):333–334. doi:10.3201/eid2002.131109

Quach C, Piche-Walker L, Platt R, Moore D (2003) Risk factors associated with severe influenza infections in childhood: implication for vaccine strategy. Pediatrics 112(3 Pt 1):e197–e201

Roberts CW, Walker W, Alexander J (2001) Sex-associated hormones and immunity to protozoan parasites. Clin Microbiol Rev 14(3):476–488

Robinson DP, Klein SL (2012) Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. Horm Behav 62(3):263–271. doi:10.1016/j.yhbeh.2012.02.023

Robinson DP, Huber SA, Moussawi M, Roberts B, Teuscher C, Watkins R, Arnold AP, Klein SL (2011a) Sex chromosome complement contributes to sex differences in Coxsackievirus B3 but not Influenza A virus pathogenesis. Biol Sex Differ 2(1):8. doi:10.1186/2042-6410-2-8

Robinson DP, Lorenzo ME, Jian W, Klein SL (2011b) Elevated 17beta-estradiol protects females from influenza a virus pathogenesis by suppressing inflammatory responses. PLoS Pathog 7(7):e1002149. doi:10.1371/journal.ppat.1002149 PPATHOGENS-D-11-00385 [pii]

Robinson DP, Hall OJ, Nilles TL, Bream JH, Klein SL (2014) 17beta-estradiol protects females against influenza by recruiting neutrophils and increasing virus-specific CD8 T cell responses in the lungs. J Virol. doi:10.1128/JVI. 02081-13

Rogers VL, Sheffield JS, Roberts SW, McIntire DD, Luby JP, Trevino S, Wendel GD Jr (2010) Presentation of seasonal influenza A in pregnancy: 2003–2004 influenza season. Obstet Gynecol 115(5):924–929. doi:10.1097/AOG.0b013e3181da0c5e

Rojas-Suarez J, Paternina-Caicedo A, Cuevas L, Angulo S, Cifuentes R, Parra E, Fino E, Daza J, Castillo O, Pacheco A, Rey G, Garcia S, Pena I, Levinson A, Bourjeily G (2014) Maternal mortality due to pandemic influenza A H1N1 2009 virus in Colombia. J Perinat Med 42(1):19–26. doi:10.1515/jpm-2013-0140

Rowe JH, Ertelt JM, Xin L, Way SS (2012) Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. Nature 490(7418):102–106. doi:10.1038/nature11462

Saito S, Nakashima A, Shima T, Ito M (2010) Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol 63(6):601–610. doi:10.1111/j.1600-0897.2010.00852.x

Schatz M, Camargo CA Jr (2003) The relationship of sex to asthma prevalence, health care utilization, and medications in a large managed care organization. Annals Allergy Asthma Immunol 91(6):553–558. doi:10.1016/s1081-1206(10)61533-5

Schatz M, Clark S, Camargo CA Jr (2006) Sex differences in the presentation and course of asthma hospitalizations. Chest 129(1):50–55

Schumacher M, Hussain R, Gago N, Oudinet JP, Mattern C, Ghoumari AM (2012) Progesterone synthesis in the nervous system: implications for myelination and myelin repair. Front Neurosci 6:10. doi:10.3389/fnins.2012.00010

Sedyaningsih ER, Isfandari S, Setiawaty V, Rifati L, Harun S, Purba W, Imari S, Giriputra S, Blair PJ, Putnam SD, Uyeki TM, Soendoro T (2007) Epidemiology of cases of H5N1 virus infection in Indonesia, July 2005-June 2006. J Infect Dis 196(4):522–527. doi:10.1086/519692

Serfling RE, Sherman IL, Houseworth WJ (1967) Excess pneumonia-influenza mortality by age and sex in three major influenza A2 epidemics, United States, 1957–58, 1960 and 1963. Am J Epidemiol 86(2):433–441

Shu Y, Yu H, Li D (2006) Lethal avian influenza A (H5N1) infection in a pregnant woman in Anhui Province, China. N Engl J Med 354(13):1421–1422. doi:10.1056/NEJMc053524

Singh AK, Cydulka RK, Stahmer SA, Woodruff PG, Camargo CA Jr (1999) Sex differences among adults presenting to the emergency department with acute asthma. Multicenter Asthma Research Collaboration Investigators. Arch Intern Med 159(11):1237–1243

Skowronski DM, Janjua NZ, Kwindt TL, De Serres G (2013) Virus-host interactions and the unusual age and sex distribution of human cases of influenza A(H7N9) in China, April 2013. Euro Surveill 18(17):20465

Smith-Bouvier DL, Divekar AA, Sasidhar M, Du S, Tiwari-Woodruff SK, King JK, Arnold AP, Singh RR, Voskuhl RR (2008) A role for sex chromosome complement in the female bias in autoimmune disease. J Exp Med 205(5):1099–1108. doi:10.1084/jem.20070850

Straub RH (2007) The complex role of estrogens in inflammation. Endocr Rev 28(5):521–574. doi:10.1210/er.2007-0001

Subbarao K, Swayne DE, Olsen CW (2006) Epidemiology and control of human and animal influenza. In: Kawaoka Y (ed) Influenza virology: current topics. Caister Academic, Norfolk, pp 229–281

Sun J, Madan R, Karp CL, Braciale TJ (2009) Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. Nat Med 15(3):277–284. doi:10.1038/nm.1929

Tamma PD, Ault KA, del Rio C, Steinhoff MC, Halsey NA, Omer SB (2009) Safety of influenza vaccination during pregnancy. Am J Obstet Gynecol 201(6):547–552. doi:10.1016/j.ajog.2009.09.034

Tate MD, Deng YM, Jones JE, Anderson GP, Brooks AG, Reading PC (2009) Neutrophils ameliorate lung injury and the development of severe disease during influenza infection. J Immunol 183(11):7441–7450. doi:10.4049/jimmunol.0902497

Tate MD, Brooks AG, Reading PC, Mintern JD (2012) Neutrophils sustain effective CD8(+) T-cell responses in the respiratory tract following influenza infection. Immunol Cell Biol 90 (2):197–205. doi:10.1038/icb.2011.26

Teilmann SC, Clement CA, Thorup J, Byskov AG, Christensen ST (2006) Expression and localization of the progesterone receptor in mouse and human reproductive organs. J Endocrinol 191(3):525–535. doi:10.1677/joe.1.06565

Uchide N, Ohyama K, Bessho T, Takeichi M, Toyoda H (2012) Possible roles of proinflammatory and chemoattractive cytokines produced by human fetal membrane cells in the pathology of adverse pregnancy outcomes associated with influenza virus infection. Mediat Inflamm 2012:270670. doi:10.1155/2012/270670

Vaillant L, La Ruche G, Tarantola A, Barboza P (2009) Epidemiology of fatal cases associated with pandemic H1N1 influenza 2009. Euro Surveill 14(33)

Vanders RL, Gibson PG, Murphy VE, Wark PA (2013) Plasmacytoid dendritic cells and CD8 T cells from pregnant women show altered phenotype and function following H1N1/09 infection. J Infect Dis 208(7):1062–1070. doi:10.1093/infdis/jit296

Villa E, Karampatou A, Camma C, Di Leo A, Luongo M, Ferrari A, Petta S, Losi L, Taliani G, Trande P, Lei B, Graziosi A, Bernabucci V, Critelli R, Pazienza P, Rendina M, Antonelli A, Francavilla A (2011) Early menopause is associated with lack of response to antiviral therapy in women with chronic hepatitis C. Gastroenterology 140(3):818–829. doi:10.1053/j.gastro.2010.12.027

Wang CS, Wang ST, Chou P (2002) Efficacy and cost-effectiveness of influenza vaccination of the elderly in a densely populated and unvaccinated community. Vaccine 20(19–20):2494–2499

Wang C, Yu H, Horby PW, Cao B, Wu P, Yang S, Gao H, Li H, Tsang TK, Liao Q, Gao Z, Ip DK, Jia H, Jiang H, Liu B, Ni MY, Dai X, Liu F, Van Kinh N, Liem NT, Hien TT, Li Y, Yang J, Wu JT, Zheng Y, Leung GM, Farrar JJ, Cowling BJ, Uyeki TM, Li L (2014) Comparison of Patients Hospitalized With Influenza A Subtypes H7N9, H5N1, and 2009 Pandemic H1N1. Clin Infect Dis. doi:10.1093/cid/ciu053

WHO (2009) Pandemic influenza A (H1N1) 2009 virus vaccine - conclusions and recommendations from the October 2009 meeting of the immunization Strategic Advisory Group of Experts. Wkly Epidemiol Rec 84(49):505–508

WHO (2013) Update on human cases of influenza at the human-animal interface, 2012. Weekly Epidemiological Record 88

WHO (2014a) Influenza update number 205. World Health Organization

WHO (2014b) WHO Global Epidemiological Surveillance Standards for Influenza. World Health Organization

WHO (2014c) WHO risk assessment of human infection with avian influenza A(H7N9) virus. World Health Organization

WHO (2014d) World: affected areas with confirmed cases of H5N1 avian influenza since 2003, status as of 24-Jan-2014 (latest available update) World Health Organization, http://gamapserver.who.int/mapLibrary/app/searchResults.aspx

Woolston WJ, Conley DO (1918) Epidemic pneumonia (spanish influenza) in pregnancy: effect in one hundred and one cases. J Am Med Assoc 71(23):1898–1899. doi:10.1001/jama.1918.02600490030008

Yang P, Pang X, Deng Y, Ma C, Zhang D, Sun Y, Shi W, Lu G, Zhao J, Liu Y, Peng X, Tian Y, Qian H, Chen L, Wang Q (2013) Surveillance for avian influenza A(H7N9), Beijing, China, 2013. Emerg Infect Dis 19(12):2041–2043. doi:10.3201/eid1912.130983

Yang S, Chen Y, Cui D, Yao H, Lou J, Huo Z, Xie G, Yu F, Zheng S, Yang Y, Zhu Y, Lu X, Liu X, Lau SY, Chan JF, To KK, Yuen KY, Chen H, Li L (2014) Avian-origin influenza A(H7N9) infection in influenza A(H7N9)-affected areas of China: a serological study. J Infect Dis 209 (2):265–269. doi:10.1093/infdis/jit430

Yu H, Gao Z, Feng Z, Shu Y, Xiang N, Zhou L, Huai Y, Feng L, Peng Z, Li Z, Xu C, Li J, Hu C, Li Q, Xu X, Liu X, Liu Z, Xu L, Chen Y, Luo H, Wei L, Zhang X, Xin J, Guo J, Wang Q, Yuan Z, Zhou L, Zhang K, Zhang W, Yang J, Zhong X, Xia S, Li L, Cheng J, Ma E, He P, Lee SS, Wang Y, Uyeki TM, Yang W (2008) Clinical characteristics of 26 human cases of highly pathogenic avian influenza A (H5N1) virus infection in China. PLoS ONE 3(8):e2985. doi:10.1371/journal.pone.0002985

Chapter 7
Sex, Gender, and Hemorrhagic Fever Viruses

Jonas Klingström and Clas Ahlm

Abstract It is estimated that more than 100 million individuals worldwide are annually infected with viruses that can cause a viral hemorrhagic fever (VHF). The pathogenesis behind various forms of VHF is generally not well understood, hampering the development of vaccines and specific treatments. Despite their importance for public health, there is with some exceptions currently a lack of safe vaccines and effective drugs. Ribavirin, an antiviral compound, is used for treatment of certain forms of VHFs, but unfortunately it has teratogenic effects and is therefore not recommended for pregnant women. In general, little is currently known regarding possible sex and/or gender differences in risk of exposure to VHFs and if there are sex differences in severity of and/or mortality from VHF. Further, little is known regarding possible sex differences in effects and side effects of the vaccines and treatments currently in use. Where data exists, it is often contradictory; for example, more cases of hantavirus infection are recognized among males than females. Seroprevalence data, however, show a more equal sex distribution, suggesting either a gender bias in case recognition, diagnoses, or both. Conversely, there might also be a sex difference in biological susceptibility to hantavirus infection. Strikingly, at present there are gaps of knowledge regarding possible sex differences in susceptibility, disease presentation, severity, and outcome in VHF. Additional clinical and epidemiological studies are needed to improve our understanding of these often fatal infectious diseases.

J. Klingström (✉)
Center for Infectious Medicine, Department of Medicine, Karolinska University Hospital Huddinge, Karolinska Institutet, Stockholm, Sweden
e-mail: Jonas.Klingstrom@ki.se

C. Ahlm
Division of Infectious Diseases, Department of Clinical Microbiology, Umeå University, Umeå, Sweden

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_7

7.1 Introduction

Viral hemorrhagic fevers (VHFs) constitute a threat for public health worldwide. This group of sometimes highly fatal infections is caused by zoonotic viruses belonging to the viral families *Arenaviridae*, *Bunyaviridae*, *Filoviridae*, and *Flaviviridae* (Table 7.1). All of these viruses are enveloped negative-stranded RNA viruses. Several of the members are also recognized as potential bioterrorism agents. The epidemiology varies, and in numerous countries these pathogens are endemic, but there are also examples of VHFs that can create large unexpected outbreaks with extensive consequences, especially in low-income countries. Common clinical features are high fever, myalgia, bleeding tendency, and sometimes kidney and/or liver involvement. Laboratory findings are characterized by increase of inflammation markers and thrombocytopenia. The pathogenesis is believed to be due to affected endothelial functions, leading to increased vascular permeability. However, the exact mechanism(s) underlying the pathogenesis in VHF in general, and for the specific forms of VHF in particular, is unknown, which hampers the development of specific treatments. Furthermore, the extent to which exposure, pathogenesis, prognosis, and treatment of VHF differ between the sexes has been grossly understudied.

7.2 Dengue Fever

Dengue virus (DENV) is a common and widely distributed virus affecting at least 100 countries. The virus is transmitted among humans through the bite of female mosquitoes of the *Aedes* genus that act as vectors. DENV is efficiently spread in cities in endemic areas as *Aedes* mosquitoes breed in small pools of water close to human dwellings. Dengue fever has increased due to an increase in the geographical range of the vectors, increased urbanization, and an increase in human population density. The World Health Organization (WHO) estimates that more than 50 million DENV infections occur yearly. Of those infected with DENV, approximately 500,000 are hospitalized every year, particularly in Asia and South America.

Dengue fever is characterized by raised temperature, headache, and muscle and joint pains accompanied with a skin rash. The infection can be asymptomatic or mild, but in a small proportion of cases, the more severe forms of dengue fever develop, termed dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Patients with DHF exhibit plasma leakage, bleeding, and thrombocytopenia, and DSS is characterized by critically low blood pressure leading to shock and severe organ impairment. There are no FDA-approved drugs for dengue fever, and consequently treatment is supportive and consists of intravenous fluid replacement (Guzmán and Kourí 2002).

Table 7.1 Overview of viral hemorrhagic fevers, distribution, vectors, and sex differences

Virus family Genus species	Disease	Distribution	Vector	Available vaccine/treatment	Reported sex differences in disease
Arenaviridae					
Arenavirus			Rodents	No/no	Not known
Lassa virus	Lassa fever,	Africa South			
Junin virus, Machupo virus, Guanarito virus	South American HF	America South America			
Bunyaviridae					
Hantavirus	HFRS/HPS	Worldwide	Rodents	Yes[a]/no	Prevalence M > F
					Mortality F > M
Phlebovirus					
Rift Valley fever virus	Rift Valley fever	Africa, Arabian Peninsula,	Mosquitoes	No[b]/no	Prevalence M > F
SFTS	Severe fever with thrombocytopenia syndrome	China	Ticks	No/no	Mortality F > M
Nairovirus					
Crimean-Congo HFV	CCHF		Ticks	No/yes	Not known
Filoviridae					
Ebola virus	Ebola hemorrhagic fever	Africa	Monkey, bat[c]	No/no	Prevalence F > M
Marburgvirus	Marburg hemorrhagic fever			No/no	Not known
Flaviviridae					
Dengue	Dengue fever DHF DSS	Tropics and subtropics worldwide	Mosquitoes	No/no	Prevalence F > M Mortality F > M
Yellow fever virus	Yellow fever	South America Africa	Mosquitoes	Yes/no	Mortality Young F > M Aged M > F

[a]A vaccine toward hantavirus is in use in China and Korea, and this vaccine is not approved by FDA
[b]Rift Valley fever vaccine for animals exists
[c]Not well known

There are four serotypes of DENV, and an infection results in neutralizing antibodies and a lifelong immunity specific to the serotype responsible for the infection. Not only does this immunity not provide cross protection for subsequent infection with another serotype, it increases the risk for the development of the more severe aspects of disease including DHF or DSS. Children in highly endemic countries are more likely than adults to be affected by these severe forms of dengue fever. This is believed to be due to the presence of non-neutralizing maternal antibodies in their circulation or a previous DENV infection during childhood (Guzmán and Kourí 2002; Whitehorn and Simmons 2011). Therefore, young children should be targeted in drug trials and dengue vaccine development.

As dengue fever is such an important emerging infection globally, it raises concern regarding age, sex, and gender differences, which have been addressed by the WHO (WHO 2007). Differences in health-seeking behavior could influence the sex ratio in hospitalized children and their treatment and may also potentially result in differences in disease outcomes in populations of male and female children (WHO 2007). Incidence rates according to sex vary in different studies. In a systematic literature search and analysis of data of dengue fever in Brazil during 2000–2010, the highest annual incidence was over 1 million cases. There were regional differences, but overall females were more likely than males to be infected by DENV, with the male/female ratio for dengue fever ranging from 0.5 to 1.1:1 (Teixeira et al. 2013).

There are also data supporting that sex differences exist in dengue fever pathogenesis. In a Vietnamese study comprising over 132,000 dengue cases, 55 % of pediatric and adult dengue fever cases were in males. In the pediatric group, girls had higher risk of developing DSS and a higher case-fatality rate than boys (Anders et al. 2011). Similarly, a recent meta-analysis of 198 studies revealed that young age (<12 years) and female sex were significantly associated with DSS (Huy et al. 2013). The increased risk of severe dengue fever and death among young girls emphasizes that further studies of possible sex differences in the pathogenesis of dengue as well as in possible gender-based bias in health-seeking behavior and clinical care are warranted.

Reports reveal an increased risk for severe dengue fever and fatal outcome during pregnancy. Pregnant women have a 3.4 times increased risk of developing severe dengue fever, DHF, and DSS than nonpregnant women, and this risk increases further with gestational age (Machado et al. 2013). Moreover, DENV also poses a risk to the fetus, as there is a risk of vertical transmission and neonatal infection (Pouliot et al. 2010). Other consequences of DENV infection are premature labor, premature birth, hemorrhage during labor, fetal death in utero, and miscarriage (Basurko et al. 2009; Adam et al. 2010). In a prospective study in Malaysia, women presenting with miscarriage were more likely to test positive for a recent DENV infection as compared with women whose pregnancies were viable (Tan et al. 2012). There is a need for more studies to verify these findings.

7.3 Yellow Fever

The disease yellow fever (YF) is caused by the yellow fever virus (YFV), present in sub-Saharan Africa and tropical regions of South America. YFV is transmitted to humans via infected female mosquitoes, mainly *Aedes* spp. in Africa and *Haemagogus* spp. in South America. *Aedes* transmit the virus directly from YFV-infected humans to humans, while *Haemagogus* mainly transmit the virus from infected monkeys to humans (Barrett et al. 2007). In cities, *Aedes aegypti* transfer YFV between humans. This mosquito is also the vector for DENV, but although the same vector carries both these viruses, YFV has in contrast to DENV not spread to Asia (Agampodi and Wickramage 2013).

The incubation period for YFV is only 3–6 days. In most cases YFV infection is asymptomatic or causes mild YF. The infection/case ratio has been estimated to be 7:1 (Monath et al. 1980). However, during epidemics, 20–50 % of the population can be infected, and around 15 % of infections result in more severe YF that ultimately leads to a fulminant VHF with a significant case-fatality rate of 20–50 %. Hence, YF causes substantial problems for the health-care systems in affected areas (Barrett et al. 2007). In a study from Brazil, old age and male sex were associated with increased risk for a lethal outcome during YF (Tuboi et al. 2007).

No specific treatment is available, and only supportive care can be given to YF patients. However, an effective live attenuated vaccine, the YF17D vaccine, exists and has been in use since the late 1930s. While the vaccine is considered safe, there can be severe side effects that sometimes are fatal (<1 case/1,000,000 vaccine doses; Nordin et al. 2013; Biscayart et al. 2014). A rare consequence of YF vaccination is YF17D vaccine-associated viscerotropic disease (YEL-AVD), which is associated with high lethality. YEL-AVD is mainly found in young females with innate immunity defects and elderly males with age-related immune senescence (Monath 2012). Lethality is higher in the young females than in the elderly males (Monath 2012). In particular women 19–34 years of age might be at higher risk than males and other age groups for YFV vaccine-associated death (Seligman 2011).

7.4 Lassa Fever

Lassa virus (LASV), a member of the *Arenaviridae*, causes Lassa fever in West Africa. The natural host for LASV is the multimammate rat, *Mastomys natalensis*. Humans are infected via inhalation and contact with virus-contaminated rodent excreta and blood, but direct human-to-human transmission via body fluids also occurs. The incubation period is normally 1–3 weeks. Lassa fever comes in a wide spectrum of symptoms, which makes it difficult to diagnose.

The estimated annual total number of Lassa fever cases ranges from 300,000 to 2,000,000, with an estimated case-fatality rate in the range of 0.25–2 % (McCormick et al. 1987; Fichet-Calvet and Rogers 2009; Falzarano and Feldmann 2013; McLay et al. 2014). It is currently not well known why certain individuals succumb to the disease while others quickly recover after a mild disease. The only available treatment is the antiviral ribavirin (McLay et al. 2014). No vaccine is available. Viremia might predict the outcome as high concentrations of LASV are associated with poor prognosis (Johnson et al. 1987; Oldstone and Campbell 2011). Lassa fever is considered a mild hemorrhagic fever, as reflected by the relatively low case-fatality rate. However, given the sheer number of annual cases, it poses a severe public health problem. In addition, in pregnant women, spontaneous abortion can occur (Jeffs 2006). In, e.g., Nigeria, nosocomial outbreaks are also a serious concern for the health-care system (Ajayi et al. 2013; Fisher-Hoch et al. 1995).

In areas where LASV is endemic, the seroprevalences in the populations are high: around 20 % of the population in Nigeria, Cöte d'Ivoire, Benin, and Ghana and over 50 % of the population in certain regions of Sierra Leone and Guinea are estimated to have been infected with LASV (Tomori et al. 1988; Gire et al. 2012).

An unusual aspect of LASV infection is that patients generally become immune suppressed (McLay et al. 2014). While macrophages and dendritic cells are infected by LASV, this does not trigger activation of innate immune cells (Baize et al. 2004). High titers of Lassa virus-specific IgG were detected in two individuals infected more than 40 years previously, suggesting that infection activates a robust, long-term antibody response (Bond et al. 2013). Deafness occurs in 15–30 % of Lassa fever patients, and in many cases this results in a permanent loss of hearing (Cummins et al. 1990). Studies on possible sex-specific patterns in the risk of being infected with LASV or on severity/mortality of Lassa fever are lacking.

7.5 Ebola Hemorrhagic Fever and Marburg Hemorrhagic Fever

Case-fatality rates can be as high as 90 % during local outbreaks of Ebola hemorrhagic fever and Marburg hemorrhagic fever. Ebolaviruses and Marburgviruses belong to the *Filoviridae* family. Ebola hemorrhagic fever is endemic in Central Africa, where three species of ebolaviruses, mainly Zaire virus (associated with case-fatality rates of 60–90 %), Sudan virus (associated with case-fatality rates of 40–60 %), and also Bundibugyo virus (associated with a case-fatality rate of 25 %), circulate and infect humans (Feldmann and Geisbert 2011). Reston virus, the only *Ebolavirus* not associated with human disease, is found in the Philippines. Marburg hemorrhagic fever is caused by Marburg virus (which is associated with

case-fatality rates of 70–85 %) and Ravn virus (Towner et al. 2009; Johnson et al. 1996). Ebola hemorrhagic fever and Marburg hemorrhagic fever are very rare diseases; in total less than 2,500 clinical cases were reported from the first known outbreak in 1967 and up to 2008 (Leroy et al. 2011). However, large outbreaks have occurred, and the local effect of these on the areas where they occur can be devastating (MacNeil and Rollin 2012). Importantly, in 2014 the so far largest Ebola hemorrhagic fever outbreak reported struck west Africa (Baize et al. 2014; Dixon and Schafer 2014), reaching for the first time a capital city showing that Ebola virus outbreaks occur over larger areas than previously believed and also that it can hit large cities.

Ebola hemorrhagic fever and Marburg hemorrhagic fever have incubation periods of around 3–13 days followed by an abrupt onset of symptoms (Kortepeter et al. 2011). Those that survive the first 2 weeks of the disease most often survive. As these diseases, like VHF in general, present with nonspecific symptoms, there is a high risk of person-to-person spread, including nosocomial transmission, before Ebola hemorrhagic fever and Marburg hemorrhagic fever are suspected and diagnosed.

It seems that women are at higher risk than men for Ebola hemorrhagic fever, likely because women are more involved in caretaking and hence are at increased risk of being exposed to Ebola virus from Ebola hemorrhagic fever patients (McElroy et al. 2014). There is an increased risk for miscarriage, and it is suspected that there is a high case-fatality rate for children of infected mothers (Feldmann and Geisbert 2011). In contrast to common beliefs, hemorrhagic manifestations are evident in less than half of the patients, and it is not associated with increased risk for lethal outcome; instead it is believed that the systemic inflammation, especially the production of pro-inflammatory cytokines, is an important factor for lethal outcome (McElroy et al. 2014). Because females typically mount higher inflammatory responses than males (see Chap. 1), this might suggest that females would suffer a worse outcome than males following infection with Ebola virus. No treatment or vaccines are available against Ebola or Marburg hemorrhagic fever. Ribavirin has no effect on filoviruses (Huggins 1989) and is therefore not recommended as a treatment. The sex of individuals should be considered when designing vaccines and treatments for these VHF viruses.

The very high case-fatality rates observed for Ebola and Marburg outbreaks in Central Africa might partly depend on the lack of good health-care systems and hospitals. An outbreak of Marburg virus in Europe in 1967, caused by imported monkeys, had a case-fatality rate of 23 %, much lower than the case-fatality rates for Marburg virus outbreaks in Central Africa, suggesting that state-of-the-art intensive care provided by well-equipped hospitals can decrease the case-fatality rate substantially (Clark et al. 2012; Feldmann and Geisbert 2011).

7.6 Crimean-Congo Hemorrhagic Fever

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease caused by CCHF virus (CCHFV), a member of the *Nairovirus* genus in the *Bunyaviridae* family. CCHF is one of the most widespread tick-borne infectious diseases in the world and has been reported in more than 30 countries in Africa, Asia, Europe, and the Middle East (Ergonul 2006; Sargianou and Papa 2013). CCHFV is a zoonotic virus, but unlike most other viruses that cause VHF, it infects a wide variety of mammal species. The natural host is the tick that also serves as the vector, transmitting the virus to humans and other mammals (Hoogstraal 1979; Bente et al. 2013). In contrast to human infection, CCHFV infection in animals is asymptomatic (Ergonul 2006). How CCHFV causes disease in humans is unknown, and why specifically humans get CCHF after CCHFV infection is also not understood (Swanepoel et al. 1989, Geisbert and Jahrling 2004). Humans are normally infected via a tick bite from the main vectors, *Hyalomma* ticks. Infection can also occur via close contact with CCHF-infected humans or via contact with infected livestock, e.g., during slaughter (Ergonul 2006). Nosocomial outbreaks occur, which is of specific concern for health-care workers (Ergonul 2006; Naderi et al. 2011).

In most cases CCHF is a mild disease, but it can be very severe, and has been associated with a case-fatality rate of up to 30 %. However, more recent studies indicate that the case-fatality rate is lower. Turkey, which has had in total 6,864 recognized CCHF cases between 2002 and 2012, has an overall annual case-fatality rate of 4–5 % (Yagci-Caglayik et al. 2014). CCHFV is classified into genetically distinct clades based on a region of the genome termed the S segment (Ergonul 2012). It is likely that specific clades have specific pathogenic properties that might be associated with the severity of infection they induce. A low- or even nonpathogenic strain of CCHFV was recently reported to circulate in Greece (Papa et al. 2014), and different strains, which might differ in their ability to cause human disease, have been reported to circulate also in Turkey (Yagci-Caglayik et al. 2014).

More males than females have been reported (Yilmaz et al. 2008; Yilmaz et al. 2009; Sisman 2013; Mofleh and Ahmad 2012; Yagci-Caglayik et al. 2014) to be diagnosed with CCHFV, but comprehensive studies are lacking, and it therefore remains to be shown if there are sex-dependent differences in the risk of CCHFV infection. The disease seems to be milder in children than adults (Tezer et al 2010; Tuygun et al. 2012; Belet et al. 2014). A total case-fatality rate of 1.1 % (2 deaths out of 179 recorded cases in patients under the age of 18 years) in Turkey (Belet et al. 2014) supports this notion. In contrast, in an Iranian study, the case-fatality rate for patients under 18 years of age (with 13 males out of 14 total cases) was 26 % (Sharifi-Mood et al. 2008), indicating that high case-fatality rates can occur also in younger age groups. Importantly, the small number of cases studied makes it difficult to draw conclusions regarding if the severity of CCHF might be age dependent.

There is no FDA-approved vaccine or treatment for CCHF. Currently CCHF patients are given supportive therapy, often together with ribavirin (Keshtkar-Jahromi et al. 2011). A vaccine, based on the CCHFV strain V42/81 propagated in suckling mouse brain, is in use in Bulgaria (Papa et al. 2011). This vaccine can induce a low level of neutralizing antibodies, but whether it protects against CCHF has not been properly addressed (Mousavi-Jazi et al. 2012). Females often develop higher neutralizing antibody responses against viral vaccines than their male counterparts (Klein et al. 2010). Greater consideration as to whether the CCHFV vaccine is more efficacious in females should be given. Although ribavirin is often administrated to CCHF patients, it is unclear if it has a beneficial effect (Keshtkar-Jahromi et al. 2011; Duygu et al. 2012). Anti-CCHFV immunoglobulin, from convalescent CCHF patients, has also been used, but as for ribavirin, studies addressing its efficacy for CCHF patients are lacking (Keshtkar-Jahromi et al. 2011). There are strong side effects of ribavirin precluding its use in pregnant women with suspected CCHF. Bradycardia was observed in ribavirin-treated children diagnosed with CCHF, indicating that treatment might worsen the outcome of the disease (Belet et al. 2014; Oflaz et al. 2013).

7.7 Hemorrhagic Fever with Renal Syndrome and Hantavirus Pulmonary Syndrome

Hantaviruses (family *Bunyaviridae*) are rodent, insectivore, and bat-borne viruses represented by more than 25 subtypes worldwide, each having a specific reservoir (Jonsson et al. 2010). Only rodent-borne hantaviruses are known to cause disease in humans. Hantavirus-infected host rodents are believed to be chronically infected and excrete the virus through saliva, urine, and feces. Human disease manifests, depending on the virus, as hemorrhagic fever with renal syndrome (HFRS) in Eurasia caused by Hantaan, Puumala, Dobrava, and Seoul viruses or hantavirus pulmonary syndrome (HPS; also called hantavirus cardiopulmonary syndrome (HCPS)) in the Americas caused by Andes virus, Sin Nombre virus, and related viruses. The incubation period in humans is long, lasting 2–3 weeks. Although symptoms of HFRS and HPS can vary, the common features of both diseases are increased vascular permeability and a vigorous immune response at the onset of the disease. The pathogenesis of hantavirus is hypothesized to be mediated by excessive pro-inflammatory and cytotoxic NK- and T-cell responses, rather than by virus replication (Björkström et al. 2011; Lindgren et al. 2011).

The case-fatality rate depends on the syndrome and, at least partly, on the virus responsible. For HFRS, case-fatality rates range from 0.4 % for Puumala virus (Hjertqvist et al. 2010) to 3 % for Hantaan and Seoul viruses in China (Zhang et al. 2010). Sin Nombre and Andes viruses have higher case-fatality rates, ranging from 30 to 40 %.

Hospital-based studies from various countries indicate that male sex is a risk factor for HFRS. In the highly endemic countries Sweden and Finland, there is a system of notification from laboratories and clinicians that makes the collection of data on sex and age very reliable. Studies comprising large cohorts of patients report the overall male/female ratio for HFRS diagnoses to be 1.52:1 in >5,000 Swedish patients (Hjertkvist et al. 2010) and 1.67:1 in >22,000 Finnish patients (Makary et al. 2010). The higher incidence of HFRS in males might reflect behavioral, cultural, and social differences that affect exposure to these viruses (e.g., hazards in male-dominated recreational and occupational activities). Only a minor proportion, around 15 % of Puumala infections, is diagnosed (Ahlm et al. 1994; Makary et al. 2010), and there might be a gender bias in the recognition of cases by the health-care system. Interestingly, seroprevalence studies have revealed that there is no statistically significant difference in the prevalence of specific IgG antibodies against HFRS-causing viruses between males and females (Ahlm et al. 1994; Mertens et al. 2009). Females might be exposed in nonoccupational peridomestic work, such as cleaning of summer cottages and sheds. Females also perform much of the work in rural and farming families which poses a risk for exposure to rodent excreta through handling of wood, hay, grain, etc. One may speculate that there might be somewhat different clinical presentations between male and female patients, with females less likely to get a clinical diagnosis perhaps due to other, hitherto less known, symptomatic presentation.

Notably, even if males had higher incidence of HFRS, the case-fatality rate was significantly higher among female HFRS patients compared with males in a large study comprising >80,000 verified cases in China (Klein et al. 2011). However, the clinical disease severity did not seem to differ by sex or age when analyzing a limited number of 221 HFRS patients (Klein et al. 2011). In Sweden, HFRS caused by the milder Puumala virus led to an increased mortality for females the first year after infection (Hjertqvist et al. 2010). Similarly, in Argentina, while males had a much higher incidence of HPS, the case-fatality rate was significantly higher among females (34 %) compared with males (21 %) (Martinez et al. 2010). Hence, it is possible that males are more likely than females to be diagnosed with HFRS/HPS, while females are more likely to succumb to the diseases than males. These discrepancies are not easily explained. Sex differences have only been addressed in a few studies. For example, in a study of German 108 HFRS patients, the male/female ratio was 3.15:1. The authors interpreted their results as "no crucial differences in the symptoms, course or severity" according to sex (Krautkrämer et al. 2013). However, females had significant lower level of plasma albumin together with higher frequency of edema and myalgia compared with males (Krautkrämer et al. 2013) which could indicate a higher level of vascular leakage, the hallmark of VHF. In a recent study with a more even sex ratio in cases, there were no differences in clinical severity according to sex (Pettersson et al. 2014). Sex differences in cytokine profile were identified during the acute phase of the disease, while no such differences were observed at follow-up during convalescence (Klingström et al. 2008). In a study of peripheral blood mononuclear cells

(PBMCs) from Puumala virus-infected patients, the clinical parameters of disease as well as estrogen receptor (ER) expression differed during the acute infection, in which PBMCs from infected males expressed elevated ERβ and PBMCs from infected females predominantly expressed ERα (Brundin et al. 2012). Sex differences have also been consistently observed in studies of rodent reservoirs for hantavirus infections, in which viral load and shedding are consistently higher in males, whereas inflammatory and antiviral immune responses are higher in females (Bagamian et al. 2013; Easterbrook and Klein 2008; Hannah et al. 2008). Animal studies suggest that there are biological sex differences in the response to hantavirus infection.

During and soon after HFRS, there is an increased risk of cardiovascular complications, such as stroke and myocardial infarction (Connolly-Andersen et al. 2014). Some people suffer from long-lasting fatigue. A possible explanation could be impaired lung function (Rasmuson et al. 2013), but also endocrine deficiencies have been noted in 18 and 56 % of Puumala patients, respectively (Stojanovic et al. 2008; Makela et al. 2010). Some of these deficiencies have been noted many years after recovery from HFRS. The physical and psychological effects of a potential hypopituitarism after HFRS warrant increased awareness. It is currently unknown if there are sex differences in long-term effects of hantavirus infection.

Women are at an increased risk of severe disease during pregnancy. For HPS, both maternal and fetal deaths may occur from 13 to 29 weeks gestation (Howard et al. 1999), but transmission to the surviving children has not been observed. For Puumala hantavirus-caused HFRS, there seems to be a risk of miscarriage when infection occurs during early pregnancy, but vertical hantavirus transmission has not been reported, at least between 14 and 38 weeks gestation (Pettersson et al. 2008; Hofmann et al. 2012). In studies of rodent reservoirs for hantaviruses, infected females do not vertically transmit virus but can transfer protective antibodies in utero and in milk (Dohmae and Nishimune 1998; Borucki et al. 2000; Kallio et al. 2006; Kallio et al. 2013).

7.8 Rift Valley Fever

Rift Valley fever virus (RVFV) is a mosquito-borne *Phlebovirus* of the *Bunyaviridae* family that causes disease in both humans and animals in endemic areas in Africa and the Arabian Peninsula. Outbreaks in East Africa have often been preceded by flooding and an increase of mosquito populations that spread the virus from infected animals to humans (Flick and Bouloy 2005). RVFV may cause large epizootics among susceptible domestic animals, e.g., cattle, sheep, and goats. Furthermore, RVFV causes abortions among infected animals, and the infection has a very high mortality in young animals.

In addition to infection via mosquitoes, animal tissues, blood, or body fluids from aborted fetuses and raw milk from infected animals may also be modes of

transmission. Depending on gender-specific work with animals and at home, women may be at an increased risk of exposure (LaBeaud et al. 2008).

Loss of animals and the ban of animal trade during outbreaks may cause devastating effects for the local economy, thereby contributing to poverty in many regions of the affected countries. The economic consequences of outbreaks especially affect the rural communities and the most vulnerable groups, i.e., women and children. Moreover, loss of livestock used for meat and dairy production may pose a threat to the nutrition of children and pregnant women in affected areas. The importance of surveillance of animals, vector control, and early case recognition among animals and humans has been proposed as part of the concept of "one health" which links approaches for animal and human health together.

In contrast to that observed for young animals and animal fetuses, human disease is often mild, with symptoms that are influenza-like. However, a more severe disease including encephalitis and retinitis may occur. In some cases, RVFV presents as a life-threatening VHF with a case-fatality rate of more than 30 % among hospitalized patients (Al-Hazmi et al. 2003). In the large outbreaks of 2007 in East Africa, more male than female cases were recognized (Nguku et al. 2010; Mohamed et al. 2010; Hassan et al. 2011). Little is known about sex differences in the clinical symptoms, complications, and outcome of RVF. There is a lack of knowledge about the risk of abortions among pregnant women. There is an indication that RVF could lead to complications in pregnant women. A study of pregnant women in Mozambique showed that RVFV-seropositive women reported a higher risk of previous miscarriages (Niklasson et al. 1987), and transmission of RVFV has been reported in a pregnant Sudanese woman (Adam and Karsany 2008). In addition, fatal neonatal infection has been reported from Saudi Arabia (Arishi et al. 2006).

7.9 Severe Fever with Thrombocytopenia Syndrome

A novel tick-borne *Bunyavirus* has recently been discovered in China (Yu et al. 2011). To identify patients with severe fever with thrombocytopenia syndrome (SFTS), a case definition was used: acute fever (temperatures of 38 °C or more) and thrombocytopenia (platelet count, <100,000 per cubic millimeter) of unknown cause (Yu et al. 2011). Of the 171 patients fulfilling the case definition of SFTS, 154 were laboratory confirmed. Of these, 56 % were women. Most of the patients were farmers, living in wooded and hilly areas, who had been working in the fields before the onset of disease. The virus was also found in ticks collected from domestic animals in the same area as the cases lived. Further studies show a case-fatality rate of 12–30 % in China, but unfortunately, the laboratory and clinical data were not analyzed separately for males and females (Deng et al. 2013; Liu et al. 2013; Wen et al. 2014; Cui et al. 2014). Liu et al. (2013) did however show that of 311 patients 55 % were females and that females might have higher case-fatality rate (Liu et al. 2013).

In a prospective study by Cui et al. 357 verified SFTSV patients were included and 57 % were females (Cui et al. 2014). In this study the case-fatality rate was higher among men in the univariate analysis, but the relation to sex was lost in a multivariate analysis (Cui et al. 2014). Notably, epidemiological investigations suggest possible person-to-person transmission with secondary cases among family members (Bao et al. 2011). Such route of transmission, earlier reported for CCHF and Andes hantaviruses (Celikbas et al. 2014; Martinez et al. 2010), is of concern for health-care workers caring for SFTSV patients.

7.9.1 Vaccines Against VHF

Although VHFs are important diseases with a large impact on public health, there is only a vaccine against YF available (Falzarano and Feldmann 2013). While the YFV virus is highly efficacious, the adverse reactions are of considerable concern. Adult females report more adverse side effects and also report more local inflammation following YF vaccination than males (Lindsey et al. 2008). Although both males and females generate equally robust antibody responses to the YF vaccination, induction of innate immune cell transcriptional responses is significantly higher in females than males immediately following vaccination (Klein et al. 2010). This live attenuated vaccine is not recommended for pregnant women due to a risk of teratogenic complications. For RVF there is a vaccine for animals, but a vaccine for humans is still lacking. Vaccines aimed against HFRS and CCHFV are available, but the efficacy can be disputed (Schmaljohn 2009; Mousavi-Jazi et al. 2012). There are no data regarding sex differences in relation to efficacy or side effects of these latter vaccines, but this should be considered in future clinical trials.

The development of human vaccines toward dengue fever, Lassa fever, CCHF, HFRS, HPS, Rift Valley fever, and Ebola and Marburg hemorrhagic fevers is ongoing, but lack of interest and resources and a lack of suitable animal models challenge the progress on these vaccines. Safe and effective vaccines against the most common VHFs, i.e., dengue fever and Lassa fever, are of highest importance for the public health in endemic areas.

7.9.2 Treatment of VHF

There are very few options available regarding antiviral treatments for VHFs, and knowledge about possible sex differences and sex bias in antiviral treatments is lacking.

The usage of ribavirin is relatively common for several VHFs, but the efficacy is not well studied. Moreover, the teratogenic effect of ribavirin is well known and raises concern when this antiviral is used for women in fertile ages. Whether the

side effects of ribavirin differ between the sexes has not been addressed. In general, females often experience more adverse reactions to drug treatments for viral infections than males, which requires more consideration in trials of antiviral drugs (see Chap. 4) (Klein 2012).

7.10 Discussion/Concluding Remarks

There are major gaps of knowledge for VHFs regarding sex differences in epidemiology, pathogenesis, clinical presentation, disease severity and outcome, and also development of vaccines and antiviral treatments. Indeed, these infections are neglected diseases in several aspects. There are both medical and economic consequences of these diseases. VHFs infect more than 100 million persons each year; but still they are seemingly neglected diseases endemic in low-income countries. VHFs may be severe and only for dengue it is estimated that 500,000 persons are hospitalized each year. The corresponding figures for other VHFs are unknown. The more severe forms of VHFs have a high case-fatality rate and frequent complications. Therefore, there is an urgent need for effective and safe treatments against VHFs in general which warrants further attention from the international community. The male predominance in some VHFs could be due to a selection bias in exposure. Alternatively, it could also be that case definitions disfavor recognition of females.

To investigate sex differences in infectious diseases in general and VHFs in particular, a comprehensive approach for studies of acute ill patients is needed. Analyses of clinical as well as pathogenic mechanisms are required to better understand a possible gender bias in disease outcome.

References

Adam I, Karsany MS (2008) Case report: Rift Valley Fever with vertical transmission in a pregnant Sudanese woman. J Med Virol 80(5):929

Adam I, Jumaa AM, Elbashir HM, Karsany MS (2010) Maternal and perinatal outcomes of dengue in PortSudan, Eastern Sudan. Virol J 7:153

Agampodi SB, Wickramage K (2013) Is there a risk of yellow fever virus transmission in South Asian countries with hyperendemic dengue? Biomed Res Int 2013:905043

Ahlm C, Linderholm M, Juto P, Stegmayr B, Settergren B (1994) Prevalence of serum IgG antibodies to Puumala virus (hemorrhagic fever with renal syndrome) in Northern Sweden. Epidemiol Infect 113(1):129–136

Ajayi NA, Nwigwe CG, Azuogu BN, Onyire BN, Nwonwu EU, Ogbonnaya LU, Onwe FI, Ekaete T, Günther S, Ukwaja KN (2013) Containing a Lassa fever epidemic in a resource-limited setting: outbreak description and lessons learned from Abakaliki, Nigeria (January-March 2012). Int J Infect Dis 17(11):e1011–e1016

Al-Hazmi M, Ayoola EA, Abdurahman M, Banzal S, Ashraf J, El-Bushra A, Hazmi A, Abdullah M, Abbo H, Elamin A, Al-Sammani e-T, Gadour M, Menon C, Hamza M,

Rahim I, Hafez M, Jambavalikar M, Arishi H, Aqeel A (2003) Epidemic Rift Valley fever in Saudi Arabia: a clinical study of severe illness in humans. Clin Infect Dis 36(3):245–252

Anders KL, Nguyet NM, Chau NV, Hung NT, Thuy TT, le Lien B, Farrar J, Wills B, Hien TT, Simmons CP (2011) Epidemiological factors associated with dengue shock syndrome and mortality in hospitalized dengue patients in Ho Chi Minh City, Vietnam. Am J Trop Med Hyg 84(1):127–134

Arishi HM, Aqeel AY, Al Hazmi MM (2006) Vertical transmission of fatal Rift Valley fever in a newborn. Ann Trop Paediatr 26(3):251–253

Bagamian KH, Towner JS, Mills JN, Kuenzi AJ (2013) Increased detection of Sin Nombre hantavirus RNA in antibody-positive deer mice from Montana, USA: evidence of male bias in RNA viremia. Viruses 5(9):2320–2328

Baize S, Kaplon J, Faure C, Pannetier D, Georges-Courbot M-C, Deubel V (2004) Lassa virus infection of human dendritic cells and macrophages is productive but fails to activate cells. J Immunol 172(5):2861–2869

Baize S, Pannetier D, Oestereich L, Rieger T, Koivogui L, Magassouba N, Soropogui B, Sow MS, Keïta S, De Clerck H, Tiffany A, Dominguez G, Loua M, Traoré A, Kolié M, Malano ER, Heleze E, Bocquin A, Mély S, Raoul H, Caro V, Cadar D, Gabriel M, Pahlmann M, Tappe D, Schmidt-Chanasit J, Impouma B, Diallo AK, Formenty P, Van Herp M, Günther S (2014) Emergence of Zaire Ebola virus disease in Guinea. N Engl J Med 371(15): 1418–1425

Bao CJ, Guo XL, Qi X, Hu JL, Zhou MH, Varma JK, Cui LB, Yang HT, Jiao YJ, Klena JD, Li LX, Tao WY, Li X, Chen Y, Zhu Z, Xu K, Shen AH, Wu T, Peng HY, Li ZF, Shan J, Shi ZY, Wang H (2011) A family cluster of infections by a newly recognized Bunyavirus in eastern China, 2007: further evidence of person-to-person transmission. Clin Infect Dis 53(12):1208–1214

Barrett AD, Monath TP, Barban V, Niedrig M, Teuwen DE (2007) 17D yellow fever vaccines: new insights. A report of a workshop held during the World Congress on medicine and health in the tropics, Marseille, France, Monday 12 September 2005. Vaccine 25(15):2758–2765

Basurko C, Carles G, Youssef M, Guindi WE (2009) Maternal and fetal consequences of dengue fever during pregnancy. Eur J Obstet Gynecol Reprod Biol 147(1):29–32

Belet N, Top A, Terzi O, Arslan HN, Baysal K, Sensoy G (2014) Evaluation of children with Crimean-Congo hemorrhagic fever in the central Blacksea Region. Pediatr Infect Dis J 33(8): e194–197

Bente DA, Forrester NL, Watts DM, McAuley AJ, Whitehouse CA, Bray M (2013) Crimean-Congo hemorrhagic fever: history, epidemiology, pathogenesis, clinical syndrome and genetic diversity. Antiviral Res 100(1):159–189

Biscayart C, Carrega ME, Sagradini S, Gentile A, Stecher D, Orduna T, Bentancourt S, Jiménez SG, Flynn LP, Arce GP, Uboldi MA, Bugna L, Morales MA, Digilio C, Fabbri C, Enría D, Diosque M, Vizzotti C (2014) Yellow fever vaccine-associated adverse events following extensive immunization in Argentina. Vaccine 32(11):1266–1272

Björkström NK, Lindgren T, Stoltz M, Fauriat C, Braun M, Evander M, Michaëlsson J, Malmberg KJ, Klingström J, Ahlm C, Ljunggren HG (2011) Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. J Exp Med 208(1):13–21

Bond N, Schieffelin JS, Moses LM, Bennett AJ, Bausch DG (2013) A historical look at the first reported cases of Lassa fever: IgG antibodies 40 years after acute infection. Am J Trop Med Hyg 88(2):241–244

Borucki MK, Boone JD, Rowe JE, Bohlman MC, Kuhn EA, DeBaca R, St Jeor SC (2000) Role of maternal antibody in natural infection of Peromyscus maniculatus with Sin Nombre virus. J Virol 74(5):2426–2429

Brundin P, Zhao C, Dahlman-Wright K, Ahlm C, Evengard B (2012) Gene expression of estrogen receptors in PBMC from patients with Puumala-virus infection. Shock 13(4):355–359

Celikbas AK, Dokuzoğuz B, Baykam N, Gok SE, Eroğlu MN, Midilli K, Zeller H, Ergonul O (2014) Crimean-Congo Hemorrhagic Fever among Health Care Workers, Turkey. Emerg Infect Dis 20(3):477–479

Clark DV, Jahrling PB, Lawler JV (2012) Clinical management of filovirus-infected patients. Viruses 4(9):1668–1686

Connolly-Andersen AM, Hammargren E, Whitaker H, Eliasson M, Holmgren L, Klingström J, Ahlm C (2014) Increased risk of acute myocardial infarction and stroke during hemorrhagic fever with renal syndrome: a self-controlled case series study. Circulation 129(12):1295–1302

Cui N, Bao XL, Yang ZD, Lu QB, Hu CY, Wang LY, Wang BJ, Wang HY, Liu K, Yuan C, Fan XJ, Wang Z, Zhang L, Zhang XA, Hu LP, Liu W, Cao WC (2014) Clinical progression and predictors of death in patients with severe fever with thrombocytopenia syndrome in China. J Clin Virol 59(1):12–17

Cummins D, McCormick JB, Bennett D, Samba JA, Farrar B, Machin SJ, Fisher-Hoch SP (1990) Acute sensorineural deafness in Lassa fever. JAMA 264(16):2093–2096

Deng B, Zhou B, Zhang S, Zhu Y, Han L, Geng Y, Jin Z, Liu H, Wang D, Zhao Y, Wen Y, Cui W, Zhou Y, Gu Q, Sun C, Lu X, Wang W, Wang Y, Li C, Wang Y, Yao W, Liu P (2013) Clinical features and factors associated with severity and fatality among patients with severe fever with thrombocytopenia syndrome Bunyavirus infection in Northeast China. PLoS ONE 8(11): e80802

Dixon MG, Schafer IJ (2014) Ebola viral disease outbreak - west Africa, 2014. MMWR Morb Mortal Wkly Rep 63(25):548–551

Dohmae K, Nishimune Y (1998) Maternal transfer of Hantavirus antibodies in rats. Lab Anim Sci 48(4):395–397

Duygu F, Kaya T, Baysan P (2012) Re-evaluation of 400 Crimean-Congo hemorrhagic fever cases in an endemic area: is ribavirin treatment suitable? Vector Borne Zoonotic Dis 12(9):812–816

Easterbrook JD, Klein SL (2008) Corticosteroids modulate Seoul virus infection, regulatory T-cell responses and matrix metalloprotease 9 expression in male, but not female, Norway rats. J Gen Virol 89(11):2723–2730

Ergonul O (2006) Crimean-Congo haemorrhagic fever. Lancet Infect Dis 6(4):203–214

Ergonul O (2012) Crimean-Congo hemorrhagic fever virus: new outbreaks, new discoveries. Curr Opin Virol 2(2):215–220

Falzarano D, Feldmann H (2013) Vaccines for viral hemorrhagic fevers–progress and shortcomings. Curr Opin Virol 3(3):343–351

Feldmann H, Geisbert TW (2011) Ebola haemorrhagic fever. Lancet 377(9768):849–862

Fichet-Calvet E, Rogers DJ (2009) Risk maps of Lassa fever in West Africa. PLoS Negl Trop Dis 3:e388

Fisher-Hoch SP, Tomori O, Nasidi A, Perez-Oronoz GI, Fakile Y, Hutwagner L, McCormick JB (1995) Review of cases of nosocomial Lassa fever in Nigeria: the high price of poor medical practice. BMJ 311(7009):857–859

Flick R, Bouloy M (2005) Rift Valley fever virus. Curr Mol Med 5(8):827–834

Geisbert TW, Jahrling PB (2004) Exotic emerging viral diseases: progress and challenges. Nat Med 10(Suppl 12):S110–S121

Gire SK, Stremlau M, Andersen KG, Schaffner SF, Bjornson Z, Rubins K, Hensley L, McCormick JB, Lander ES, Garry RF, Happi C, Sabeti PC (2012) Epidemiology. Emerging disease or diagnosis? Science 338(6108):750–752

Guzmán MG, Kourí G (2002) Dengue: an update. Lancet Infect Dis 2(1):33–42

Hannah MF, Bajic VB, Klein SL (2008) Sex differences in the recognition of and innate antiviral responses to Seoul virus in Norway rats. Brain Behav Immunol 22(4):503–516

Hassan OA, Ahlm C, Sang R, Evander M (2011) The 2007 Rift Valley fever outbreak in Sudan. PLoS Negl Trop Dis 5(9):e1229

Hjertqvist M, Klein SL, Ahlm C, Klingstrom J (2010) Mortality rate patterns for hemorrhagic fever with renal syndrome caused by Puumala virus. Emerg Infect Dis 16(10):1584–1586

Hofmann J, Führer A, Bolz M, Waldschläger-Terpe J, Meier M, Lüdders D, Enders M, Oltmann A, Meisel H, Krüger DH (2012) Hantavirus infections by Puumala or Dobrava-Belgrade virus in pregnant women. J Clin Virol 55(3):266–269

Hoogstraal H (1979) The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J Med Entomol 15(4):307–417
Howard MJ, Doyle TJ, Koster FT, Zaki SR, Khan AS, Petersen EA, Peters CJ, Bryan RT (1999) Hantavirus pulmonary syndrome in pregnancy. Clin Infect Dis 29(6):1538–1544
Huggins JW (1989) Prospects for treatment of viral haemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. Rev Infect Dis 11(Suppl 4):S750–S761
Huy NT, Van Giang T, Thuy DHD, Kikuchi M, Hien TT, Zamora J, Hirayama K (2013) Factors associated with dengue shock syndrome: a systematic review and meta-analysis. PLoS Negl Trop Dis 7(9):e2412
Jeffs B (2006) A clinical guide to viral haemorrhagic fevers: Ebola, Marburg and Lassa. Trop Doct 36(1):1–4
Johnson KM, McCormick JB, Webb PA, Smith ES, Elliott LH, King IJ (1987) Clinical virology of Lassa fever in hospitalized patients. J Infect Dis 155(3):456–464
Johnson ED, Johnson BK, Silverstein D, Tukei P, Geisbert TW, Sanchez AN, Jahrling PB (1996) Characterization of a new Marburg virus isolated from a 1987 fatal case in Kenya. Arch Virol 11:101–114
Jonsson CB, Figueiredo LTM, Vapalahti O (2010) A Global Perspective on Hantavirus Ecology, Epidemiology, and Disease. Clin Microbiol Rev 23(2):412–441
Kallio ER, Poikonen A, Vaheri A, Vapalahti O, Henttonen H, Koskela E, Mappes T (2006) Maternal antibodies postpone hantavirus infection and enhance individual breeding success. Proc Biol Sci 273(1602):2771–2776
Kallio ER, Henttonen H, Koskela E, Lundkvist A, Mappes T, Vapalahti O (2013) Maternal antibodies contribute to sex-based difference in hantavirus transmission dynamics. Biol Lett 9(6):20130887
Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S (2011) Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral Res 90(2):85–92
Klein SL (2012) Sex differences in prophylaxis and therapeutic treatments for viral diseases. Handb Exp Pharmacol 214:499–522
Klein SL, Jedlicka A, Pekosz A (2010) The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis 10(5):338–349
Klein SL, Marks MA, Li W, Glass GE, Fang LQ, Ma JQ, Cao WC (2011) Sex differences in the incidence and case fatality rates from hemorrhagic fever with renal syndrome in China, 2004–2008. Clin Infect Dis 13(12):1414–1421
Klingström J, Lindgren T, Ahlm C (2008) Sex-dependent differences in plasma cytokine responses to hantavirus infection. Clin Vaccine Immunol 13(5):885–887
Kortepeter MG, Bausch DG, Bray M (2011) Basic clinical and laboratory features of filoviral hemorrhagic fever. J Infect Dis 204(Suppl 3):S810–S816
Krautkrämer E, Grouls S, Urban E, Schnitzler P, Zeier M (2013) No gender-related differences in the severity of nephropathia epidemica, Germany. BMC Infect Dis 13:457
LaBeaud AD, Muchiri EM, Ndzovu M, Mwanje MT, Muiruri S, Peters CJ, King CH (2008) Interepidemic Rift Valley fever virus seropositivity, Northeastern Kenya. Emerg Inf Dis 14 (8):1240–1246
Leroy EM, Gonzalez JP, Baize S (2011) Ebola and Marburg haemorrhagic fever viruses: major scientific advances, but a relatively minor public health threat for Africa. Clin Microbiol Infect 17(7):964–976
Lindgren T, Ahlm C, Mohamed N, Evander M, Ljunggren HG, Björkström NK (2011) Longitudinal analysis of the human T cell response during acute hantavirus infection. J Virol 85 (19):10252–10260
Lindsey NP, Schroeder BA, Miller ER, Braun MM, Hinckley AF, Marano N, Slade BA, Barnett ED, Brunette GW, Horan K, Staples JE, Kozarsky PE, Hayes EB (2008) Adverse event reports following yellow fever vaccination. Vaccine 26(48):6077–6082

Liu W, Lu QB, Cui N, Li H, Wang LY, Liu K, Yang ZD, Wang BJ, Wang HY, Zhang YY, Zhuang L, Hu CY, Yuan C, Fan XJ, Wang Z, Zhang L, Zhang XA, Walker DH, Cao WC (2013) Case-fatality ratio and effectiveness of ribavirin therapy among hospitalized patients in china who had severe fever with thrombocytopenia syndrome. Clin Infect Dis 57 (9):1292–1299

Machado CR, Machado ES, Rohloff RD, Azevedo M, Campos DP, de Oliveira RB, Brasil P (2013) Is pregnancy associated with severe dengue? A review of data from the Rio de Janeiro surveillance information system. PLoS Negl Trop Dis 7(5):e2217

MacNeil A, Rollin PE (2012) Ebola and Marburg hemorrhagic fevers: neglected tropical diseases? PLoS Negl Trop Dis 6(6):e1546

Makary P, Kanerva M, Ollgren J, Virtanen MJ, Vapalahti O, Lyytikäinen O (2010) Disease burden of Puumala virus infections, 1995–2008. Epidemiol Infect 138(10):1484–1492

Makela S, Jaatinen P, Miettinen M, Salmi J, Ala-Houhala I, Huhtala H, Hurme M, Pörsti I, Vaheri A, Mustonen J (2010) Hormonal deficiencies during and after Puumala hantavirus infection. Eur J Clin Microbiol Infect Dis 29(6):705–713

Martinez VP, Bellomo CM, Cacace ML, Suarez P, Bogni L, Padula PJ (2010) Hantavirus pulmonary syndrome in Argentina, 1995–2008. Emerg Infect Dis 16(12):1853–1860

McCormick JB, Webb PA, Krebs JW, Johnson KM, Smith ES (1987) A prospective study of the epidemiology and ecology of Lassa fever. J Infect Dis 155(3):437–444

McElroy AK, Erickson BR, Flietstra TD, Rollin PE, Nichol ST, Towner JS, Spiropoulou CF (2014) Ebola hemorrhagic fever: novel biomarker correlates of clinical outcome. J Infect Dis 210(4):558–566

McLay L, Liang Y, Ly H (2014) Comparative analysis of disease pathogenesis and molecular mechanisms of New World and Old World arenavirus infections. J Gen Virol 95(1):1–15

Mertens M, Wölfel R, Ullrich K, Yoshimatsu K, Blumhardt J, Römer I, Esser J, Schmidt-Chanasit J, Groschup MH, Dobler G, Essbauer SS, Ulrich RG (2009) Seroepidemiological study in a Puumala virus outbreak area in South-East Germany. Med Microbiol Immunol 198 (2):83–91

Mofleh J, Ahmad Z (2012) Crimean-Congo haemorrhagic fever outbreak investigation in the Western Region of Afghanistan in 2008. East Mediterr Health J 18(5):522–526

Mohamed M, Mosha F, Mghamba J, Zaki SR, Shieh WJ, Paweska J, Omulo S, Gikundi S, Mmbuji P, Bloland P, Zeidner N, Kalinga R, Breiman RF, Njenga MK (2010) Epidemiologic and clinical aspects of a Rift Valley fever outbreak in humans in Tanzania, 2007. Am J Trop Med Hyg 83(Suppl 2):22–27

Monath TP (2012) Review of the risks and benefits of yellow fever vaccination including some new analyses. Expert Rev Vaccines 11(4):427–448

Monath TP, Craven RB, Adjukiewicz A, Germain M, Francy DB, Ferrara L, Samba EM, N'Jie H, Cham K, Fitzgerald SA, Crippen PH, Simpson DI, Bowen ET, Fabiyi A, Salaun JJ (1980) Yellow fever in the Gambia, 1978–1979: epidemiologic aspects with observations on the occurrence of orungo virus infections. Am J Trop Med Hyg 29(5):912–928

Mousavi-Jazi M, Karlberg H, Papa A, Christova I, Mirazimi A (2012) Healthy individuals' immune response to the Bulgarian Crimean-Congo hemorrhagic fever virus vaccine. Vaccine 30(44):6225–6229

Naderi HR, Sarvghad MR, Bojdy A, Hadizadeh MR, Sadeghi R, Sheybani F (2011) Nosocomial outbreak of Crimean-Congo haemorrhagic fever. Epidemiol Infect 139(6):862–866

Nguku PM, Sharif SK, Mutonga D, Amwayi S, Omolo J, Mohammed O, Farnon EC, Gould LH, Lederman E, Rao C, Sang R, Schnabel D, Feikin DR, Hightower A, Njenga MK, Breiman RF (2010) An investigation of a major outbreak of Rift Valley fever in Kenya: 2006–2007. Am J Trop Med Hyg 83(Suppl 2):5–13

Niklasson B, Liljestrand J, Bergström S, Peters CJ (1987) Rift Valley fever: a sero-epidemiological survey among pregnant women in Mozambique. Epidemiol Infect 99 (2):517–522

Nordin JD, Parker ED, Vazquez-Benitez G, Kharbanda EO, Naleway A, Marcy SM, Molitor B, Kuckler L, Baggs J (2013) Safety of the yellow Fever vaccine: a retrospective study. J Travel Med 20(6):368–373

Oflaz MB, Kucukdurmaz Z, Guven AS, Karapinar H, Kaya A, Sancakdar E, Deveci K, Gul I, Erdem A, Cevit O, Icagasioglu FD (2013) Bradycardia seen in children with Crimean-Congo hemorrhagic fever. Vector Borne Zoonotic Dis 13(11):807–811

Oldstone MBA, Campbell KP (2011) Decoding arenavirus pathogenesis: essential roles for alpha-dystroglycan-virus interactions and the immune response. Virology 411(2):170–179

Papa A, Papadimitriou E, Christova I (2011) The Bulgarian vaccine Crimean-Congo haemorrhagic fever virus strain. Scand J Infect Dis 43(3):225–229

Papa A, Sidira P, Larichev V, Gavrilova L, Kuzmina K, Mousavi-Jazi M, Mirazimi A, Ströher U, Nichol S (2014) Crimean-congo hemorrhagic Fever virus, Greece. Emerg Infect Dis 20 (2):288–290

Pettersson L, Boman J, Juto P, Evander M, Ahlm C (2008) Outbreak of Puumala virus infection, Sweden. Emerg Infect Dis 14(5):808–810

Pettersson L, Thunberg T, Rocklöv J, Klingström J, Evander M, Ahlm C (2014) Viral load and humoral immune response in association with disease severity in Puumala hantavirus-infected patients-implications for treatment. Clin Microbiol Infect 20(3):235–241

Pouliot SH, Xiong X, Harville E, Paz-Soldan V, Tomashek KM, Breart G, Buekens P (2010) Maternal dengue and pregnancy outcomes: a systematic review. Obstet Gynecol Surv 65 (2):107–118

Rasmuson J, Lindqvist P, Sörensen K, Hedström M, Blomberg A, Ahlm C (2013) Cardiopulmonary involvement in Puumala hantavirus infection. BMC Infect Dis 13:501

Sargianou M, Papa A (2013) Epidemiological and behavioral factors associated with Crimean-Congo hemorrhagic fever virus infections in humans. Expert Rev Anti Infect Ther 11 (9):897–908

Schmaljohn C (2009) Vaccines for hantaviruses. Vaccine 27(Suppl 4):D61–D64

Seligman SJ (2011) Yellow fever virus vaccine-associated deaths in young women. Emerg Infect Dis 17(10):1891–1893

Sharifi-Mood B, Mardani M, Keshtkar-Jahromi M, Rahnavardi M, Hatami H, Metanat M (2008) Clinical and epidemiologic features of Crimean-Congo hemorrhagic fever among children and adolescents from southeastern Iran. Pediatr Infect Dis J 27(6):561–563

Sisman A (2013) Epidemiologic features and risk factors of Crimean-Congo hemorrhagic fever in Samsun province, Turkey. J Epidemiol 23(2):95–102

Stojanovic M, Pekic S, Cvijovic G, Miljic D, Doknic M, Nikolic-Djurovic M, Micic D, Hrvacevic R, Nesic V, Popovic V (2008) High risk of hypopituitarism in patients who recovered from hemorrhagic fever with renal syndrome. J Clin Endocrinol Metab 93 (7):2722–2728

Swanepoel R, Gill DE, Shepherd AJ, Leman PA, Mynhardt JH, Harvey S (1989) The clinical pathology of Crimean-Congo hemorrhagic fever. Rev Infect Dis 11(Suppl 4):S794–S800

Tan PC, Soe MZ, Si Lay K, Wang SM, Sekaran SD, Omar SZ (2012) Dengue infection and miscarriage: a prospective case control study. PLoS Negl Trop Dis 6(5):e1637

Teixeira MG, Siqueira JB Jr, Ferreira GL, Bricks L, Joint G (2013) Epidemiological trends of dengue disease in Brazil (2000–2010): a systematic literature search and analysis. PLoS Negl Trop Dis 7(12):e2520

Tezer H, Sucakli IA, Sayli TR, Celikel E, Yakut I, Kara A, Tunc B, Ergonul O (2010) Crimean-Congo hemorrhagic fever in children. J Clin Virol 48(3):184–186

Tomori O, Fabiyi A, Sorungbe A, Smith A, McCormick JB (1988) Viral hemorrhagic fever antibodies in Nigerian populations. Am J Trop Med Hyg 38(2):407–410

Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A, Swanepoel R, Paddock CD, Balinandi S, Khristova ML, Formenty PB, Albarino CG, Miller DM, Reed ZD, Kayiwa JT, Mills JN, Cannon DL, Greer PW, Byaruhanga E, Farnon EC, Atimnedi P, Okware S, Katongole-Mbidde E, Downing R, Tappero JW, Zaki SR, Ksiazek TG, Nichol ST, Rollin PE

(2009) Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS Pathog 5(7):e1000536

Tuboi SH, Costa ZG, da Costa Vasconcelos PF, Hatch D (2007) Clinical and epidemiological characteristics of yellow fever in Brazil: analysis of reported cases 1998–2002. Trans R Soc Trop Med Hyg 101(2):169–175

Tuygun N, Tanir G, Caglayik DY, Uyar Y, Korukluoglu G, Cenesiz F (2012) Pediatric cases of Crimean-Congo hemorrhagic fever in Turkey. Pediatr Int 54(3):402–406

Wen HL, Zhao L, Zhai S, Chi Y, Cui F, Wang D, Wang L, Wang Z, Wang Q, Zhang S, Liu Y, Yu H, Yu XJ (2014) Severe fever with thrombocytopenia syndrome, Shandong Province, China, 2011. Emerg Infect Dis 20(1):1–5

Whitehorn J, Simmons CP (2011) The pathogenesis of dengue. Vaccine 29(42):7221–7228

WHO (2007) Addressing sex and gender in epidemic-prone infectious diseases. WHO, Geneva. http://www.who.int/csr/resources/publications/SexGenderInfectDis.pdf. Accessed 24 Feb 2014

Yagci-Caglayik D, Korukluoglu G, Uyar Y (2014) Seroprevalence and risk factors of Crimean-Congo hemorrhagic fever in selected seven provinces in Turkey. J Med Virol 86(2):306–314

Yilmaz GR, Buzgan T, Torunoglu MA, Safran A, Irmak H, Com S, Uyar Y, Carhan A, Ozkaya E, Ertek M (2008) A preliminary report on Crimean–Congo haemorrhagic fever in Turkey. Euro Surveill 13(33):18953

Yilmaz GR, Buzgan T, Irmak H, Safran A, Uzun R, Cevik MA, Torunoglu MA (2009) The epidemiology of Crimean–Congo hemorrhagic fever in Turkey, 2002–2007. Int J Infect Dis 13 (3):380–386

Yu XJ, Liang MF, Zhang SY, Liu Y, Li JD, Sun YL, Zhang L, Zhang QF, Popov VL, Li C, Qu J, Li Q, Zhang YP, Hai R, Wu W, Wang Q, Zhan FX, Wang XJ, Kan B, Wang SW, Wan KL, Jing HQ, Lu JX, Yin WW, Zhou H, Guan XH, Liu JF, Bi ZQ, Liu GH, Ren J, Wang H, Zhao Z, Song JD, He JR, Wan T, Zhang JS, Fu XP, Sun LN, Dong XP, Feng ZJ, Yang WZ, Hong T, Zhang Y, Walker DH, Wang Y, Li DX (2011) Fever with thrombocytopenia associated with a novel Bunyavirus in China. N Engl J Med 364(16):1523–1532

Zhang YZ, Zou Y, Fu ZF, Plyusnin A (2010) Hantavirus infections in humans and animals, China. Emerg Infect Dis 16(8):1195–1203

Chapter 8
Gender Issues in Tuberculosis

Anna Thorson

Abstract Tuberculosis (TB) is an infectious disease, caused by *Mycobacterium tuberculosis*. When aggregated by sex, global TB notification rates show more male cases of TB reported than female. Cases of smear-positive pulmonary TB also are reportedly higher for males than females. Tuberculosis bacteria usually infect the lungs, but infection can occur in any other organ. For reasons that are not completely clear, a higher proportion of extra-pulmonary TB occur among females than males. TB ranks as the second leading cause of death in humans from a single infectious disease globally, after HIV, and thus remains a global health concern although it is in fact a curable disease. Gender differences in health-seeking behaviors, including male-dominant utilization of TB diagnostic and screening services as well as the extent to which women have to negotiate their healthcare seeking, likely impact male-female differences in TB. Differences in the sensitivity of conventional molecular diagnostic tests as well as in the prevalence of multi drug-resistant TB can result in sex- and gender-based differences in treatment of TB. The role of co-infection with other pathogens, including HIV, as well as how biological differences between the sexes affect the prevalence and outcome of TB require additional consideration.

8.1 Introduction

Tuberculosis (TB) is an infectious disease, caused by *Mycobacterium tuberculosis*. Members of the *Mycobacteriaceae* group of organisms, which can cause human disease, include *M. tuberculosis*, *Mycobacterium africanum*, *Mycobacterium avium*, *Mycobacterium bovis*, *Mycobacterium microti*, and *Mycobacterium canettii*. *M. bovis* was responsible for about 6 % of all human tuberculosis deaths in Europe before the introduction of milk pasteurization (Lawn and Zumla 2011). Tuberculosis bacteria usually infect the lungs, but infection can occur in any other organ. The disease (i.e., active tuberculosis) occurs in a minority of those who are infected.

A. Thorson (✉)
Department of Public Health Sciences, Karolinska Institutet Stockholm, Sweden
e-mail: Anna.Thorson@ki.se

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_8

In most cases, the bacterium enters a dormant state, termed latent tuberculosis infection (LTBI), in which it remains for a period lasting from weeks to many years. TB ranks as the second leading cause of death in humans from a single infectious disease globally, after the human immunodeficiency virus (HIV) (WHO 2013), and thus remains a global health concern although it is in fact a curable disease.

TB is spread via airborne droplets, produced during coughing or breathing. A number of studies show that the risk of transmission is related to the infectivity of the primary case, the duration and proximity of contact with the primary case, and being in an enclosed space with the primary case (Fox et al. 2011). Consequently, those who spend the most time with the patient during their period of infectivity (usually weeks to months before diagnosis) are at the highest risk of infection (Fox et al. 2011).

A study on social mixing conducted in South Africa showed that among persons living in a township with a high incidence of TB, most contacts were generated by sharing air in households (25 %), while in the workplace and community buildings, sharing air generated 8 and 6 % contacts, respectively (Wood et al. 2012).

8.2 Epidemiology of Disease

Millions of people, especially those residing in the 22 TB high-burden countries (HBCs) (Fig. 8.1a), get TB each year. In 2012, approximately 8.6 million new TB cases were reported, while 1.3 million people died from the disease (including 320,000 people with HIV coinfections (WHO 2013)). Most of the reported TB cases and deaths are among men (Borgdorff et al. 2000; Dye 2006); however, TB is an enormous health problem also among women. Of the estimated 8.6 million new TB cases worldwide in 2012, 2.9 millions were females (WHO 2013). Most of the estimated number of cases reported for 2012 occurred in Asia (58 %) and the African region (27 %). The five countries with the largest number of incident cases were India (2.0–2.4 million), China (0.9–1.1 million), South Africa (0.4–0.6 million), Indonesia (0.4–0.5 million), and Pakistan (0.3–0.5 million) (WHO 2013); see Fig. 8.1b.

Since 1990, mortality due to TB has decreased by 45 %, and incidence rates are also declining in the six WHO regions (WHO 2013). Between 1995 and 2012, 56 million people in countries that adopted the directly observed treatment (DOT)/Stop TB Strategy were successfully treated for TB, while 22 million lives were saved (Glaziou et al. 2011).

The global epidemic trends of TB indicate that the United Nations (UN), Millennium Development Goal (MDG) 6, Target 8 of reducing the incidence of TB by 2015 has been achieved. However, the MDG framework "Stop TB Partnership" that aims to halve TB prevalence and death rates globally remains a challenge (Dye 2006; WHO 2013). This has led to increased prominence of the post-2015 agenda on the development framework to reduce the disease burden from HIV/AIDS, TB, malaria, neglected tropical diseases, and noncommunicable diseases (WHO 2013). TB is a disease closely associated with poverty and social

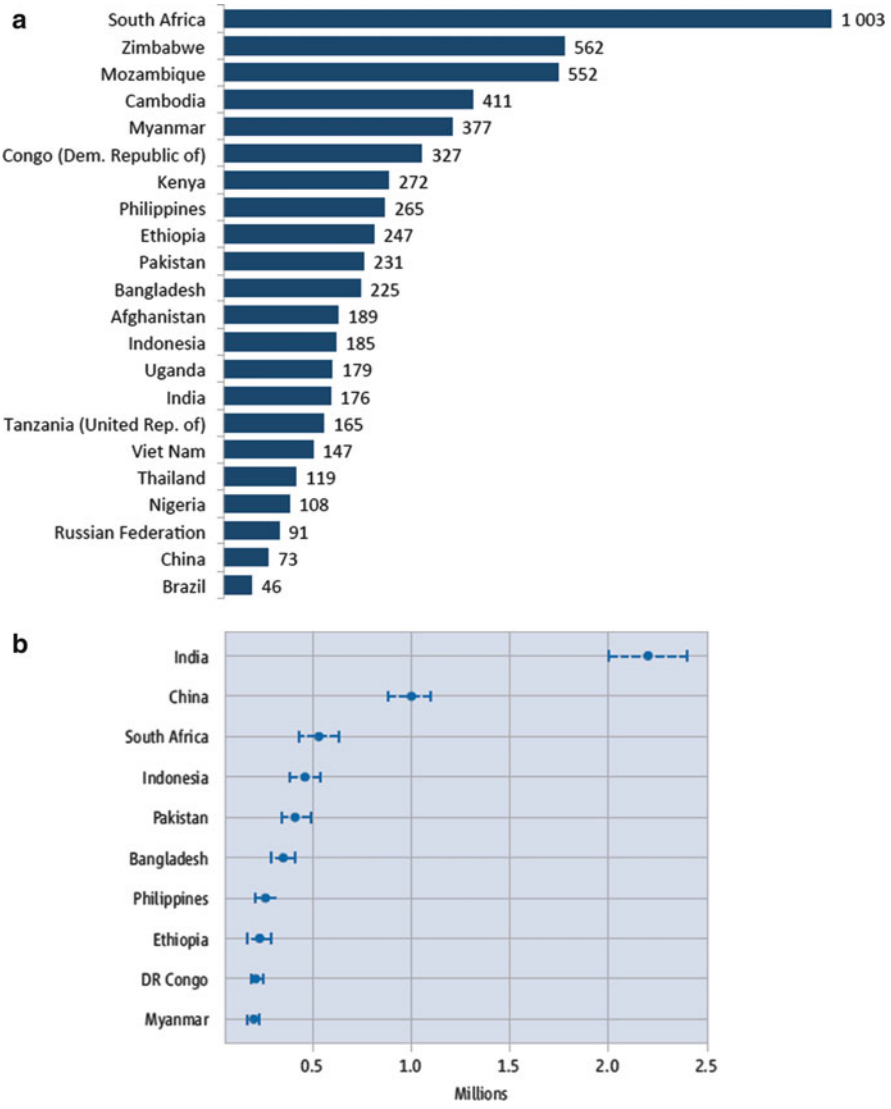

Fig. 8.1 (**a**) Incidence (rate per 100,000 population) of TB in the 22 high-burden countries, 2012 (*Source* Kaiser Family Foundation, www.GlobalHealthFacts.org, based on WHO global report on TB, 2013). (**b**) Incidence (in absolute numbers) of TB in the top ten burdened countries (*Source* WHO, Global Tuberculosis Report, 2013)

inequities. To build on the goals of the 2015 agenda of eradicating TB in affluent countries, together with reaching significant goals in low- and middle-resource settings where the largest disease burden remains, will require significant efforts to reduce inequities in access to prevention, care, and treatment of TB. Gender is a structural cause of inequity, influencing the complexity and burden of TB to individuals and families. Prevailing gender inequities globally highlight the need

of addressing TB from a perspective that examines not only biological sex differences but also the variety of different situations and contexts faced by men and women.

8.2.1 Consequences of Infection and TB Disease

M. tuberculosis has evolved elaborate survival mechanisms in human beings that allow it to remain in a clinically latent state (Ottenhoff et al. 2005). Upon infection with *M. tuberculosis*, individuals may develop either clinical disease (primary progressive TB) or latent TB infection (LTBI), which may reactivate at a later date and result in active TB disease anywhere in the body. Treatment of people with LTBI can significantly reduce their risk of reactivation. The risk of developing an active disease in the first 5 years following primary infection is greater than the risk in subsequent years (Andrews et al. 2012; Musellim et al. 2005). The risk of developing a clinical disease is determined by multiple microbiological, immunological, social, and cultural factors (Seddon et al. 2013), including sex-mediated differences (Rhines 2013). HIV infection and its negative effects on the immune response are the single most important cause of developing an active TB disease, which also shows sex-specific patterns (see Chap. 5).

8.3 Sex and Gender Differences in TB Infection and Progression to Active Disease

Tuberculin surveys carried out during the 1950s and early 1960s show a rather uniform pattern, with an equal prevalence of infection among boys and girls until the age of 15 years, after which the prevalence in males begins to exceed that in females (Dolin 1998). Surveys of TB infection are dependent on the tuberculin (purified protein derivative [PPD]) testing. There is evidence of differences in tuberculin reactivity between men and women with active tuberculosis, related to differences in immune responses to the bacterium. A study conducted in Japan in the 1990s reported the tuberculin skin test to be less effective among women (Diwan and Thorson 1999; Watkins and Plant 2006a, b). Hudelson (1996) suggested that gender differences may contribute to the risk of infection due to societal norms, for example, in settings where men work outside homes and are exposed to social contacts (Hudelson 1996). However, these norms differ in different settings and do not adequately explain why the PPD test would be less effective in women than men.

To what extent and in what way tuberculin reactivity following latent infection differs between men and women, how gender/sex characteristics interact with the well-known limited tuberculin reaction following HIV infection and age-dependent changes in sex hormones (e.g., testosterone levels in males or estrogen levels in

females) are not well understood. Hence, there are inherent difficulties in drawing conclusions about disease from tuberculin surveys of TB infection/latent TB, due to several potential sources of misclassification of infection status.

The number of longitudinal studies analyzing the risk of tuberculin-positive individuals progressing to active tuberculosis is very limited. Those that exist show in turn a higher risk of progression among females of reproductive age (Rieder 1999; UNAIDS 2007) and among men who smoke (Balasubramanian et al. 2004). The male to female ratio in disease progression in a study population in south India dropped from 2.7 to 1.2 after exclusion of men who were smokers or abused alcohol. An ecological study indicated that one-third of the sex/gender difference in tuberculosis is explainable by male smoking (Watkins and Plant 2006a, b).

8.4 Sex and Gender Differences in the Incidence of Disease Manifestations

8.4.1 Pulmonary Tuberculosis: Notification Rates and General Pattern of Pulmonary TB

When aggregated by sex, global TB notification rates show more male cases of TB reported than female. Incidence estimates of pulmonary TB, produced annually by the World Health Organization (WHO) global TB program, rely on notification rates together with a combination of available (national) prevalence or the occasional incidence survey. The earlier estimates based on annual risk of infection studies have to some extent been abandoned by the global TB research community. High-burden countries consistently report more male than female cases, while there are large variations in magnitude, sex, age, and disease patterns (WHO 2013). The HIV pandemic has had a profound impact on the epidemiology of tuberculosis, which has become the most important cause of death among those infected with HIV in sub-Saharan Africa. In this region, where HIV rates among young women can be 3–6 times higher than among men of the same age group, the corresponding high rate of coinfection with HIV and *M. tuberculosis* is having a significant impact on the epidemiology of tuberculosis. TB is also among the top killers of women of reproductive age, and 510,000 women died from TB in 2013. While the reported case notification rates show a predominance of male cases globally, around half of TB deaths among those coinfected with HIV were in women in 2013. Some 90 % of these deaths occurred in Africa, where TB is estimated to have claimed more female lives than male lives (WHO 2013).

By contrast, male–female differences in reported cases of TB in many of the former Soviet Union countries are greater than expected, with the largest disease burden by far occurring in men. There are gender discrepancies in well-known risk behaviors associated with risk of developing active TB such as alcohol, substance

and tobacco abuse, which may explain the high numbers of reported cases in males in these settings.

Providing population-based estimates of TB prevalence or incidence is complex and resource intensive, requiring large sample sizes and accurate laboratory analyses. Consequently, very few studies or national surveys have been able to address or verify the observed gender differences in TB notification rates. The reported gender differences have hence been debated, and hypotheses for the underlying phenomenon have been proposed. Some have argued that the lower rates of diagnosed and reported TB among women compared with men, in some contexts, may be attributed to failure to diagnose suspected cases among women or might reflect differences in access to adequate healthcare among women and men (Hudelson 1996; Holmes et al. 1988, 1998; Diwan and Thorson 1999; Thorson et al. 2000). Evidence from population-based studies in some contexts supports this hypothesis (Thorson 2003).

Other researchers have argued that differences in TB prevalence between males and females represent real epidemiology differences and not merely a product of differences in healthcare. A meta-analysis of 29 surveys conducted in 14 countries consistently found more TB cases among males than females. Since the findings of this study indicated a strong male bias in both TB prevalence and notification rates, the authors concluded that a difference in access to healthcare was ruled out as a confounding factor (Borgdorff et al. 2000). Another review highlighted the existence of more cases among men also in settings where there were no significant differences in health-seeking behavior between the sexes (Rhines 2013).

Additionally, empirical evidence suggests a complex situation, where context seems to interplay with biological risks for differentiating risks of TB in men and women. A large community randomized household survey with equal participation between males and females in Bangladesh reported that the male to female ratio in TB cases was 3:1 (Hamid Salim et al. 2004). In a multicenter case–control study in West Africa, the male to female ratio was 2.03:1 despite approximately equal sex ratios in household contacts and community controls (Lienhardt et al. 2005). Likewise, a national prevalence survey from Vietnam in 2006–2007 showed a ratio of 5:1 of PTB among men versus women (Hoa et al. 2010).

In a recent hospital-based study conducted among patients with suspected TB in Southeast Ethiopia, there was no significant difference between men and women in the prevalence of smear-positive pulmonary TB (PTB) among suspected cases (Tulu et al. 2014), while another retrospective study, which included record reviews from Ethiopia, indicated that more males than females had PTB (Mekonnen 2014). Likewise in Mexico, a study using molecular epidemiological techniques found a higher (58 %) incidence of PTB in males than in females, 32 cases per 100,000 person years and 20 cases per 100,000 person years, respectively. The reason for more cases among males was attributed to both reactivation and latent infection and recent TB transmission. Cases of reactivated TB were also higher in males than females (24 vs. 15 cases per 100,000 person years, respectively). No significant sex differences were observed on the median time interval between onset of symptoms and diagnosis or between the median time interval between onset of symptoms and

treatment (Jiménez-Corona et al. 2006). Other studies have found that PTB occurs within the first 3 years of primary contact; thereafter, the risk decreases (Iseman 2000). Many studies from various settings globally have examined notification rates or hospital data and showed a predominance of male cases (Muvunyi et al. 2010).

In conclusion, there is a consistent general pattern of a predominance of male PTB cases, increasing with age. The evidence from population-based studies challenging this finding in some contexts may, however, suggest that deeper gender analyses are needed to assess local needs in most high-burden settings where coverage goals are not met.

8.4.2 Extra-pulmonary Tuberculosis

In general, about 80 % of all cases of tuberculosis involve the lungs, but in populations with a high prevalence of HIV infection, extra-pulmonary tuberculosis is relatively more frequent (Rieder 1999; Haas 2000). Extra-pulmonary TB (EPTB) refers to all forms of TB that are not pulmonary. In addition to a general spread that may occur at primary sites of infection, miliary or disseminated TB involves sites of reactivation for ETPB, including, but not limited to, lymph nodes, bones, joints, soft tissue, and genitourinary system (Musellim et al. 2005; Sreeramareddy et al. 2008), and may vary according to geographic location and population (Sreeramareddy et al. 2008). A study on socio-demographic factors contributing to the development of EPTB reported that females born in Asia or North Africa were at a higher risk of developing EPTB than males (Calihol et al. 2005).

Findings from a study in Turkey with an approximately 1:1 ratio of females to males demonstrated a significant difference in the sex ratio among EPTB and PTB cases (Musellim et al. 2005). The presentation of EPTB versus PTB cases was 74 and 34 %, respectively, among females compared with males. Females had a higher (3.69-fold) risk of developing EPTB, and the risk increased 5 years after the primary contact with a known case of PTB (Musellim et al. 2005). In Nepal, comparing PTB and EPTB showed that young age and female gender were associated with EPTB relative to PTB. The likelihood of developing EPTB was 1.5 times higher among females than males (Sreeramareddy et al. 2008). The gender differences observed in this study were consistent with studies conducted in other settings (Holmes et al. 1998; Martinez et al. 2000; Chan-Yeung et al. 2002). Some explanations of these findings have been suggested to be a result of gender differences in exposures to TB infection and prevalence of susceptibility risk factors.

In a retrospective review of EPTB cases in a hospital in Kabul, Afghanistan, females were more likely to be diagnosed with EPTB than males at a ratio of 2.03:1. The average duration of symptoms before presentation was 14.8 months for males and 18.0 months for females. In this study, more females than males reported symptoms lasting 1 year or longer prior to diagnosis. Overall, lymph nodes followed by the central nervous system were the most common sites of EPTB (Fader et al. 2010).

Although several studies have revealed a higher proportion of EPTB among women than men, the reason for this is unknown. In a WHO study in Bangladesh, Columbia, India, and Malawi (WHO 2006), it was found that women presented to the clinic with a greater diversity of nonspecific physical symptoms, and it was concluded that "health care professionals should be trained to consider the possibility of TB in females patients presenting with more atypical symptoms."

8.4.3 Concurrency of EPTB and PTB

Concurrency with PTB and EPTB is another challenge in TB management and treatment where gender differences have been observed. EPTB is a more common manifestation among immune-compromised hosts, such as people living with HIV. A recent population-based study in Taiwan investigated the association of gender with concurrent PTB plus EPTB (Lin et al. 2013). In this study, women >45 years had a higher likelihood of concurrent disease than men. However, in younger patients there were no significant differences between the sexes. Three independent factors that influenced concurrent PTB and EPTB infections in this population were gender, HIV coinfection, and cough ≥3weeks. The authors suggested that because male–female differences in the concurrency of PTB and EPTB are only evident in patients 45 years and above, a possible explanation is that the immune systems of older females are less able than females below 45 years of age to contain bacilli locally in the lung parenchyma. In addition, hormonal factors may play a role in the greater susceptibility of older females to EPTB (Lin et al. 2013). In conclusion, very little is known about the underlying immunological mechanisms that shift TB disease toward PTB versus ETB and its relation to gender, with or without the presence of other infectious diseases, such as HIV.

8.4.4 Confounding Factors

There exists an increased risk of developing active TB following infection, from 7 to 10 % annually in individuals who concurrently have LTBI and untreated HIV infection, as compared to a 10 % lifetime risk for those with LTBI who are not HIV infected. This risk is reduced with antiretroviral therapy for HIV but is still higher than that in HIV-negative persons with LTBI (CDC 2013). TB/HIV coinfection has a significant social and economic impact, as young adults are the predominant age group affected. In sub-Saharan Africa where HIV rates among young females can be 3–6 times higher than among males of the same age group, the corresponding high rate of coinfection with HIV and *M. tuberculosis* is having a significant impact on the epidemiology of tuberculosis (Rhines 2013). In Malawi, for example, females in the reproductive age range (i.e., 15–45) make up a major proportion of those who are coinfected (Glynn et al. 2004). In addition, the stigma associated with

TB is particularly evident in regions with a high prevalence of HIV infection. The generally high prevalence of HIV among females and the relationship between HIV infection and TB risk may render females vulnerable to resistant TB. To date, little research has been done on the sex- and gender-related factors affecting risks of coinfection (see Chap. 5).

Additional risk factors for TB have been studied and reported to include malnutrition, smoking, alcoholism, overcrowding, silicosis, diabetes, and poverty (Narasimhan et al. 2013). An ecological study indicated that one-third of the sex/gender difference in tuberculosis is explainable by male smoking (Watkins and Plant 2006a, b). Recent microbiological studies suggest that mechanisms between the risk of TB and male sex are less clear and likely complex (Nhamoyebonde and Leslie 2014). In the 20 HBCs for which data were available for the WHO 2013 TB report, the median male to female ratio was 1.8:1 (WHO 2013).

There is also evidence suggesting that TB and parasitic diseases are more prevalent in males than females, most likely reflecting differences in exposure to the pathogen as well as susceptibility to infection (van Lunzen and Altfeld 2014). Other studies have suggested that the disparities in TB prevalence between the sexes are affected by sex hormones, genetic factors, and nutritional status that may minimize the severity of TB in females (Neyrolles and Quintanna-Murci 2009; Forssborhm et al. 2008).

8.4.5 Effects of Pregnancy

Diagnostic approaches to TB are similar for HIV-infected pregnant females and HIV-uninfected and nonpregnant females. Major challenges in the diagnosis of TB in HIV-infected pregnant females have been described and include nonspecific TB symptoms, or absent symptoms, weight gain due to physiological changes as a result of the pregnancy, and an overlap between TB and HIV symptoms (Loto and Awowole 2012). Among pregnant women with TB disease, those infected with HIV are less likely than those not infected with HIV to be sputum smear positive (Gounder et al. 2011). Several studies that have examined the relation between TB and pregnancy or the post-partum period leave more questions than answers, since results are contradictory (Festenstein and Grange 1999). In South Africa, the high burden of both HIV and TB contributes to high maternal mortality rates (Martin and Black 2012). The prevalence of TB in HIV-infected pregnant females in South Africa is similar to that in the general population, approximately 795/100,000 (WHO 2012). Whether pregnant women, in general, are at an increased risk of severe TB has not been conclusively demonstrated to date.

8.5 Differences in Health-Seeking Behavior Among Men and Women

8.5.1 Health-Seeking Behavior and Gender

Studies from several settings show that significantly more men than women access tuberculosis diagnostic and screening services (Boeree et al. 2004). A study carried out in Eastern Nepal in the early 1980s showed that when cases were actively sought by household visits, 46 % of the detected cases were females, compared to only 28 % in the self-referral group. Factors such as stigma and discrimination may play a role in these differences in case finding. In a study of the population-based prevalence of sputum smear-positive TB in a rural district in Vietnam, a male to female ratio of 0.7:1 was found suggesting an underdiagnosis of TB cases in women (Thorson 2003; Thorson et al. 2004).

Health seeking has been defined using the following concepts: symptom recognition (i.e., to recognize a symptom as a health problem), sick role (i.e., the patients consider themselves as "sick" and are ready to take an action), lay referral (i.e., discussions and guidance by people within their own social networks), and treatment action (Ngamvithayapong et al. 2001).

Several gender and health studies in high-income countries have shown that women use healthcare facilities more often than men (Kandrack et al. 1991; Verbrugge 1989). This has been accredited to various factors, including a higher actual morbidity among women, women of reproductive age having closer contact with the healthcare system through antenatal and mother and child, care and the female gender role allowing women to acknowledge ill health to a higher degree than the male gender role (Verbrugge 1989; Kandrack et al. 1991; Doyal 1995). The situation is quite different in low-income settings where women may face more barriers to adequate healthcare because they have less access to financial resources and less decision-making power of their own. Their workload is also often heavier than that of men leaving less time to care for themselves. Being responsible for the health of the family, women often have to put their own needs in the background, with resources being spent on the children or husband (Vlassoff and Bonilla 1994; AbouZahr et al. 1996).

A qualitative study in Ethiopia documented a case in which a woman's husband prevented her from seeking help (Cambanis et al. 2005). Access to adequate healthcare cannot be taken for granted for either men or women and is also closely related to socio-economic status for both sexes. In India, women are found to underreport morbidity and are said to practize a "culture of silence" regarding their illnesses (Rangan and Uplekar 1998; Fochsen et al. 2006). The causes for the delays in TB diagnosis among women include poor economic status, and clinical disease status, and complexities in referral and diagnostic procedure at different districts (Wang et al. 2007).

A study of TB in Bangladesh showed that more men than women sought public healthcare for respiratory complaints, which was interpreted as representing a

possible barrier in access to healthcare for these women (Watkins and Plant 2006a, b). A study in urban Vietnam showed that female TB patients were more likely than males to use a private provider in their health-seeking process (Lonnroth et al. 2001). A population-based study of women and men with prolonged cough found that significantly more women than men had used low-quality providers of care such as drug sellers or private practitioners, whereas men were more likely to have used the national healthcare system with direct access to hospital care (Thorson et al. 2000). In India, although women were more likely than men to first consult a private provider, the median patient delay (the time from symptom recognition to the patient seeks healthcare) was similar among male and female TB patients (Balasubramanian et al. 2004). In a WHO study conducted in Bangladesh, Columbia, India, and Malawi, fewer women than men were identified as suspected TB cases in India and Bangladesh, with an equal number in Malawi and more women than men in Columbia.

In a qualitative study of TB patients in Vietnam, stigma and fear of social consequences were found to influence healthcare seeking by women to a greater extent than by men (WHO 2006). These factors were considered to contribute to symptom denial and a preference for private or other nonpublic providers (Johansson et al. 2000). Similar findings emerged from a study based on in-depth interviews with TB patients in Pakistan, where women were more likely than men to report difficulty in obtaining adequate TB treatment because of restrictions of their movements and a general unwillingness on the part of the household decision makers to pay for their treatment (Khan et al. 2000). Tuberculosis-related stigma was also reported as being greater for women than for men, and unmarried women, in particular, were afraid to announce that they had the disease for fear of not being able to get married. In India, a significantly higher proportion of women than men faced social stigmatization or rejection because of their illness, with 21 % of women and 14 % of men feeling inhibited to discuss their illness with friends or family (WHO 2006). Women were also more likely to need someone to accompany them to directly observed treatment (DOT) than men (WHO 2006; Khan et al. 2000).

A study exploring cultural concepts of TB and gender among a general population of individuals without tuberculosis in rural India found that emotional and social symptoms were more frequently reported for females and included arranged marriages, social isolation, and inability to care for children and family. In contrast, job loss and reduced income were regarded as most troubling for the males (Atre et al. 2004). In another study in Vietnam, women with cough were shown to have less knowledge than men about the medical characteristics of TB, and this in turn resulted in them seeking care from less qualified providers. Traditional beliefs about TB seem to be strongly related to stigma and a lack of knowledge about the characteristics of the disease, which could be related to disempowerment regarding the perceived available choices for seeking healthcare.

In Zambia, there is conflicting evidence regarding factors associated with a long patient delay in seeking healthcare among patients with cough; old age and severe disease were linked to a long delay, whereas gender, stigma, and less knowledge

about TB characteristics were not associated with delays in health-seeking behaviors (Godfrey-Fausett et al. 2002). This opposes earlier findings from the same country where being female and having a low educational level were factors linked to a longer delay among TB patients (Needham et al. 2001). In general, evidence in relation to patient delay is conflicting from different parts of the world, but there is a growing body of literature indicating that a longer provider delay (i.e., the time from a patient seeks healthcare to that he/she gets an accurate diagnosis) for women has been suggested in several studies (Haas 2000; WHO 2006; Needham et al. 2001; Pronyk et al. 2001; Yamasaki-Nakagawa et al. 2001).

In some countries, the greater tendency for women to initially contact a traditional healer for TB may explain the longer doctor's delay. In the other settings, delays occurred after contact with the national healthcare system, and the patient delay was not significantly different between women and men in these studies. In Sarawak, Malaysia, being female was significantly associated with patient delay (Chang and Esterman 2007), whereas no association was found with provider delay.

In Bangladesh, women who present with respiratory symptoms are less likely to undergo sputum smear examination than men (Watkins and Plant 2006a, b). In Malawi, more men than women submitted sputum specimens for diagnosis of TB, although there were no data on the relative number of those seeking healthcare that had symptoms suggestive of this disease. In the population-based study from Vietnam, among those with cough, women had been asked to provide a sputum sample at the hospital significantly less often than men, a difference which persisted when corrections were made for the presence and duration of symptoms (Lonnroth et al. 2001). In the WHO study in Bangladesh, Columbia, India, and Malawi (WHO 2006), more women than men dropped out during the process of diagnosis.

Little is known about the actual mechanisms involved in creating a longer provider delay, including reasons for a lower access to diagnostic investigations for female suspected TB cases. The patient–doctor encounter is likely to be of importance not only for patient satisfaction and adherence but also for a successful health outcome. In an interview study with healthcare providers in Vietnam, male doctors expressed the opinion that female TB patients are more difficult to diagnose due to communication problems, whereas female doctors did not perceive any gender-related problems in this respect (Thorson and Johansson 2004).

The preference of patients with symptoms suggestive of TB, principally women but also men, to opt for care within the private sector should be systematically addressed in low-income countries. The use of unregulated providers needs to be recognized as a gender issue, as has been shown in several low-income countries (Uplekar et al. 2001; Rangan and Uplekar 1998; Fochsen et al. 2006; Thorson et al. 2000). Special attention to gender-related issues is thus needed in order to improve healthcare seeking and case detection of TB, especially among women. In the WHO study in Bangladesh, Columbia, India, and Malawi, experiences with semi-active case finding (i.e., targeted actions to increase diagnosis of TB, such as screening contacts of diagnosed patients, or community outreach initiatives) in Bangladesh had provided good results in terms of reducing patient delay to TB diagnosis although it had no effects on provider delay (WHO 2006).

In Peruvian shantytowns, the TB program was perceived not to be gender discriminatory and provided equal diagnostic and treatment care to men and women. This contrasted with stereotypical gender roles in the broader community context and a commonly expressed belief among patients and healthcare workers that female health inherently has a lower priority than male health. This belief was principally associated with the dominant role of men in the household economy and limited employment for women in this setting. However, women generally were more likely than men to report adverse psychosocial and economic consequences of TB diagnosis more. Of note in this study was a common perception that TB care of women was of secondary importance to that of men. This reflected the influence of societal gender values despite apparent gender equality in care provision. The study concluded that the greatest opportunities for improving women's access to TB care involve improving social, political, and economic structures more than TB program modification (Onifade et al. 2010).

Evidences from different countries therefore suggest that women have to negotiate their healthcare seeking to a greater extent than men, often because of a combination of sociocultural factors, such as responsibility for the household and the children and more limited access to resources, whereas the concerns of men are more straightforward and concern livelihood activities as the prime cause of unnecessary patient delays (WHO 2006; Thorson 2003). While women are disadvantaged in terms of access in several settings, men also may face difficulties accessing diagnosis and treatment for TB, and a better understanding of the barriers they face is also necessary.

It is often suggested that women face less healthcare access barriers than men in some settings, because women have access to health facilities through standardized care targeted to pregnant women, mothers, and children. However, a study in India found that women visited health facilities for immunizations and other reasons associated with the wellbeing of their children, and not primarily for their own health (Balasubramanian et al. 2000, 2004). In other studies conducted in different settings, factors found to associate with low case detection among women were sociocultural and low socio-economic status of women (Johansson et al. 2000; Sanchez-Perez et al. 2002), poor knowledge of identifying and reporting TB symptoms, and healthcare practitioners' delay in making a TB diagnosis (Long et al. 2002; Mfinanga et al. 2008). In contrast, a Mexican study found that there were no differences in access to healthcare services between men and women as has been suggested by some studies in developing countries. Further, lower socio-economic status among women was not a barrier to PTB screening, diagnosis, treatment, and access to healthcare; in fact, women used these services more frequently than men (Jiménez-Corona et al. 2006). In a study to determine PTB cases among women with a cough in Tanzania, risk factors associated with a smear-positive result included attending more than one visit to any facility prior to diagnosis. In this study, reasons for women's prior visit to health facilities were not reported (Ngadaya et al. 2009).

A systematic review of qualitative studies assessing gender-related barriers and delays in accessing TB diagnostic and treatment services reported that men faced

work-related financial and physical barriers and community stigma while women experienced financial and physical dependence (Krishnan et al. 2014). Other barriers such as those experienced at the level of care, low literacy levels, and stigma affected both men and women much the same way. Also striking in this review was the absence of clear differences between urban and rural contexts or between types of health facilities.

8.5.2 Diagnosis of TB Among Men and Women

Conventional lab tests for the diagnosis of TB are sputum smear microscopy and bacterial culture where the reference standard is usually based on cultured specimens (WHO 2013). In 2008, WHO recommended rapid molecular tests (line probe assays or LPAs) for detection of rifampicin-resistant TB (RR-TB) and MDR-TB using positive sputum specimens. In 2010, WHO endorsed the first rapid molecular test that can simultaneously test for pulmonary TB and rifampicin resistance, making this a more sensitive and much better test than smear microscopy (WHO 2013).

In 2006, it was reported that existing diagnostic methods could detect up to 60 % of TB cases and as such there was a call to strengthen laboratory networks to aid detection of all forms of TB (Onyebujoh 2006). In 2004, countries reported 1.4 million smear-positive cases in men, but only 775,000 in women (Dye 2006). Also, studies from Bulgaria, Bangladesh, and Malawi reported that proportionally more men than women among those who submitted a sputum specimen were found to be positive for acid-fast bacilli on microscopical examination (WHO 2006; Tsankova et al. 2014). Differences in sensitivity of the diagnostic may reflect a difference in prevalence of active TB, but these differences also may be due to sex-specific differences in physiological characteristics of TB lesions or to gender-related differences when seeking diagnosis. The latter includes sociocultural restrictions for women against coughing and spitting, making it less likely that women will produce good sputum samples. Studies in Vietnam have shown that men were given sputum examinations more often than women. The same studies also found health system factors to be a barrier to diagnosis, in which women with pulmonary TB were diagnosed on average 2 weeks later than men because of delays from the healthcare provider (Thorson and Diwan 2001; Thorson 2003). In Pakistan, lower smear positivity in women than in men was mainly a function of poor-quality specimen submission. Smear positivity in women was increased substantially by provision of brief additional instructions (Khan et al. 2007). In some instances, women have poorer access to diagnostic facilities (Dye 2006).

The use of DNA fingerprinting techniques to study clustering of pulmonary TB cases in the Netherlands led to the conclusion that females with pulmonary TB generated fewer new incident cases than males (Borgdorff et al. 2000). This study also indicated that males with pulmonary TB were positive on sputum smear examination more often than females. These findings imply that in this setting,

sputum smear microscopy for diagnosing pulmonary TB has a lower sensitivity among females than among males.

It has been suggested that chest radiology findings differ between males and females with TB due to sex differences in their immune responses to the bacterium (Bothamley 1998). In a study in Turkey, female TB patients had a higher frequency of lower lung field involvement, a finding that is usually regarded as quite uncommon in post-primary disease. In Vietnam, on the other hand, there were no differences in lung field involvement, but significantly more males than females had pleurisy and miliary patterns of disease on chest radiology (Thorson et al. 2007). Further, despite claims that females may be more difficult to diagnose due to poorer-quality sputum samples (Khan et al. 2007), male bias was still observed in studies that relied on radiographic diagnosis, a technique that excludes these confounding factors (Nhamoyebonde and Leslie 2014; Kivihya-Ndugga et al. 2005).

The overall contribution to the gender bias in tuberculosis case detection is difficult to assess (Nhamoyebonde and Leslie 2014). There seems to be no consensus on what possibly drives the differences between the sexes. Dye cites poorer access to diagnostic facilities among women (Dye 2006); however, a meta-analysis of 29 surveys conducted in 14 countries found a concordant strong male bias in both notification rates and prevalence rates, suggesting that differences in notification rates may be largely due to real epidemiological differences and not to differential access to healthcare (Borgdorff et al. 2000).

More recently, biological mechanisms (e.g., involvement of sex hormones, sex-related genetic background, and genetic regulations and metabolism, among other factors) have also been cited as a possible explanation for TB susceptibility differences between men and women (Neyrolles and Quintanna-Murci 2009). A study conducted in South Africa suggested that taking into account biological differences may help us gain a better understanding of the immune responses that are necessary for control of TB (Nhamoyebonde and Leslie 2014). While others suggest that sex and gender differences are linked to the real epidemiological differences between the sexes, both in exposure to the bacterium and in susceptibility to development of active disease, others believe that the nature of gender studies in TB is conflicting and there is a need for more detailed epidemiology data (references).

Long-term cough and sputum production are key features for suspecting TB. A study of symptoms among 757 men and 270 women with smear-positive pulmonary TB in Vietnam showed that at the time of diagnosis, fewer women than men reported the symptoms of cough, sputum production, and hemoptysis (Long et al. 2002; Haas 2000). At follow-up after 1 month of treatment, more women than men had recovered from their symptoms of cough and sputum production. Also observed was a delay in the diagnosis of PTB (by a medical practitioner), which was significantly associated with the absence of a cough and sputum expectoration in the patient (Long et al. 2002). A Zambian study found that female sex, lower education, more than six instances of health-seeking encounters, outpatient diagnosis of tuberculosis, and visiting a private doctor or traditional healer were

significantly associated with late diagnosis (Needham et al. 2001). In Bangladesh, compared with males, females experienced longer delays at various stages of the clinical process of help seeking for TB (Karim et al. 2007).

8.6 Differences in the Outcome of TB Treatment Among Women and Men

A Ugandan study investigated gender-related differences in the presentation and 1 year outcomes of HIV-infected adults with PTB. The results showed that while differences existed between men and women with HIV-associated PTB at presentation, the outcomes at 1 year after the initiation of TB treatment were similar (Nsubuga et al. 2002). Another study conducted in West Bengal, India, found that among the new smear-positive patients, 89.4 % of females were cured compared to 85.8 % of males which was significant statistically (Mukherjee et al. 2012).

8.6.1 Treatment Adherence and Social Consequences of Tuberculosis

Adherence to TB treatment has been reported and described as a complex behavioral issue influenced by factors such as gender and the impact of HIV/AIDS (Munro et al. 2007), but the specific impact of gender (Diwan and Thorson 1999) still warrants more research and reporting. Gender diffences exist in rates of adherence to treatment, with the fear and stigma associated with TB having a greater impact on women than on men, often placing them in an economically or socially precarious position. The health and welfare of children is closely linked to that of their mothers, and TB in women can have serious repercussions for families and households (Hudelson 1996).

The stigma associated with tuberculosis appears to be both substantial and universal and is described in various cultural contexts, although the form that it takes may vary from region to region, such as associations with HIV disease where this is prevalent. The social consequences of stigma often persist even when tuberculosis has been successfully cured: accounts from India, Bangladesh, and Malawi show that despite their disease being cured, women experience problems getting married. Women and men seem to experience the impact of stigma differently, though the psychological and social consequences are harsh for both genders (WHO 2006; Johansson and Winkvist 2002).

Therefore, there is a growing realization of the need to assess gender-related and other factors affecting adherence for both men and women. In the Hunan province of China, for example, interventions have been implemented to improve health disparities; however, the gender disparity remains (Chen et al. 2014). In the WHO

four-country study, psychological and emotional symptoms of distress were reported by a large majority of TB patients (WHO 2006), and these were related to stigma, discrimination, and rejection by the family. It is essential that health information and education draws a distinction between reasonable precautions to minimize contagion and the creation of unnecessary fears of tuberculosis, thereby endeavoring to reduce the stigma associated with the disease (WHO 2006).

Treatment adherence in the WHO four-country study showed a higher dropout rate among males in all four countries, and the same was found in the south India study. The financial impact of illness and hospitalization among males may be the prime cause of their lower adherence rates, as well as difficulties in reaching clinics during opening times (WHO 2006; Balasubramanian et al. 2004).

Studies in Mexico, India, and Taiwan also showed poorer treatment outcomes among males (Jiménez-Corona et al. 2006; Uplekar et al. 2001; Feng et al. 2012). The Taiwan prospective observational study suggests that male gender is associated with older age, more comorbidities, and worse treatment outcomes.

Insufficient knowledge and individual cost during treatment were reported as main obstacles to adherence among men, while sensitivity to interaction with health staff and stigma in society were reported as the main obstacles among women (Johansson et al. 1999). Fear of being associated with TB may also lead to reluctance to receive treatment as it becomes more or less obvious to anyone in the community that the patient is being treated for this disease, and this may in turn lead to delays in following the referral chain. These factors seem important for female TB patients as they may face particular constraints to daily healthcare contacts, such as lack of access to child care, to transport or to accessing money for transport (even when treatment may be free), or requiring permission from their husbands to access healthcare (Johansson et al. 2000).

A systematic review of qualitative research identified four major factors that interact to affect TB treatment as structural factors, including poverty and gender discrimination, the social context, health service factors, and personal factors (Munro et al. 2007). This review reported that adherence to TB treatment appeared to be facilitated where patients understood the importance of completing treatment. Patients' beliefs about the efficacy of treatment and the validity of diagnostic tests may impact on adherence. Other studies reviewed reported patients' desire to be cured as a motivator for adherence. Generally, female patients were perceived as more motivated which is similar to most studies on health-seeking behaviors between men and women; however, some studies reported a relationship between pregnancy and nonadherence in women. Though the systematic reviews included studies limited in terms of quality and foci, they are useful in providing a useful synthesis of views from a broad range of stakeholders (patients, caregivers, and healthcare providers) and are useful in taking forward the discussion of appropriate interventions (Munro et al. 2007).

8.6.2 Treatment Medications

The most common resistance mechanism to the first-line drugs by *M. tuberculosis* is by alteration of the target leading to insufficient binding of the drug. This arises as a result of chromosomal gene mutations. Second-line drugs, for example, fluoroquinolones, are available for the treatment of resistant TB. However, there has been reported development of drug resistance by the TB bacterial strains, which results in inefficiency of most of these drugs (Walzl et al. 2005). A study conducted in South Africa found MDR tuberculosis to be more prevalent than had been previously realized in this setting. XDR tuberculosis had been transmitted to HIV-coinfected patients and was associated with high mortality (Gandhi et al. 2006) warranting priority attention for HIV and TB integrated activities. A prospective study on prevalence of and risk factors for drug resistance among 1,278 patients in eight countries between 2005 and 2008 showed that in addition to being resistant to isoniazid and rifampicin, 49 % of patient isolates were also resistant to ethambutol and streptomycin. About 43, 13, and 7 % were resistant to, at least one second-line drug, fluoroquinolones, and extensively drug-resistant (XDR) TB (Dalton et al. 2012). The strongest risk factor for XDR-TB was previous treatment with a second-line injectable drug. Female sex was another significant risk factor (Dalton et al. 2012). Similarly a study from Ethiopia identified female sex as a significant risk factor for MDR. MDR-TB and XDR-TB are currently the greatest causes of ineffective TB therapy and have led to an increasing problem in many parts of the world especially developing countries.

8.7 Future Directions

The impact of sex and gender on the diagnosis, prognosis, and treatment of TB is still an emerging research field and presents an opportunity for continued lessons and learning. Much of the research that is discussed above has contributed to some understanding of the dynamics of managing TB; however, there is an opportunity to advance the field further. There are still significant knowledge gaps in the complex process of biological or immunological vulnerability to MTB, in relation to gender. The unequal distribution of MDR and XDR as identified in a handful of studies needs careful continued investigations to assess issues related to adherence as well as sex-specific differences in host response.

TB continues to be a major public health urgency. The global arena needs a holistic approach to research, where advancing research into improved point-of-care diagnostics and new first- and second-line drugs should be paired with efforts to reduce access barriers to diagnosis and treatment for men and women alike. Like pointed out in the post-2015 agenda, support to patients with TB need not only correct medical investigations but also schemes for social protection. From a gender perspective, research into TB household's as well as individual patient's needs in

relation to best practice provision of support will provide possibilities of further disentangling the complexity of diminishing the disease burden of TB.

References

Abouzahr C, Vlassoff C, Kumar A (1996) Quality health care for women: a global challenge. Health Care Women Int 17:449–467

Andrews JR, Noubary F, Walensky RP, Cerda R, Losina E, Horsburgh CR (2012) Risk of progression to active tuberculosis following reinfection with Mycobacterium tuberculosis. Clin Infect Dis 54:784–791

Atre SR, Kudale AM, Morankar SN, Rangan SG, Weiss MG (2004) Cultural concepts of tuberculosis and gender among the general population without tuberculosis in rural Maharashtra, India. Trop Med Int Health 9:1228–1238

Balasubramanian VN, Oommen K, Samuel R (2000) DOT or not? Direct observation of antituberculosis treatment and patient outcomes, Kerala State, India. Int J Tuberc Lung Dis 4:409–413

Balasubramanian R, Garg R, Santha T, Gopi PG, Subramani R, Chandrasekaran V, Thomas A, Rajeswari R, Ananadakrishnan S, Perumal M, Niruparani C, Sudha G, Jaggarajamma J, Frieden TR, Narayanan PN (2004) Gender disparities in tuberculosis: report from a rural DOTS programme in south India. Int J Tuberc Lung Dis 8:323–332

Boeree MJ, Harries AD, Godschalk P, Demast Q, Upindi B, Mwale A, Nyirenda TE, Banerjee A, Salaniponi FM, Boeree MJ, Harries AD, Godschalk D et al (2004) Gender differences in relation to sputum submission and smear- positive pulmonary tuberculosis in Malawi. Int J Tuberc Lung Dis 4:882–884

Borgdorff MW, Nagelkerke NJD, Dye C, Nunn P (2000) Gender and tuberculosis: a comparison of prevalence surveys with notification data to explore sex differences in case detection. Int J Tuberc Lung Dis 4:123–132

Bothamley G (1998) Sex and gender in the pathogenesis of infectious tuberculosis: a perspective from immunology, microbiology and human genetics. Nordic School of Public Health, Göteborg

Calihol J, Decludt B, Che D (2005) Sociodemographic factors that contribute to the development of extrapulmonary tuberculosis were identified. J Clin Epidemiol 58:1066–1071

Cambanis A, Yassin MA, Ramsay A, Squire SB, Arbide I, Cueva LE (2005) Rural poverty and delayed presentation to tuberculosis service in Ethiopia. Trop Med Int Health 10:330–335

CDC (2013) Latent tuberculosis infection: a guide for primary health care providers. U.S. Department of Health and Human Services Centers for Disease Control and Prevention National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention Division of Tuberculosis Elimination, Atlanta, GA

Chang CT, Esterman A (2007) Diagnostic delay among pulmonary tuberculosis patients in Sarawak, Malaysia: a cross- sectional study. Rural Remote Health 7

Chan-Yeung M, Noertjojo K, Chan SL, Tam CM (2002) Sex differences in tuberculosis in Hong Kong. Int J Tuberc Lung Dis 6:11–18

Chen M, Kwaku AB, Chen Y, Huang X, Tan H, Wen SW (2014) Gender and regional disparities of tuberculosis in Hunan, China. Int J Equity Health 13:32

Dalton T, Cegielski P, Akkslip S et al (2012) Prevalence of and risk factors for resistance to second-line drugs in people with multidrug-resistant tuberculosis in eight countries: a prospective cohort study. Lancet 380:1406–1417

Diwan VK, Thorson A (1999) Sex, gender and tuberculosis. Lancet 353:1000–1001

Dolin P (1998) Tuberculosis epidemiology from a gender perspective. In: Diwan VK, Thorson A, Winkvist A (eds) Gender and tuberculosis. Nordic School of Public Health, Göteborg, pp 29–40
Doyal L (1995) In sickness and in health, what makes women sick. Macmillan, London
Dye C (2006) Global epidemiology of tuberculosis. Lancet 367:938–940
Fader T, Parks J, Khan NU, Manning R, Stokes S, Nasir NA (2010) Extrapulmonary tuberculosis in Kabul, Afghanistan: a hospital-based retrospective review. Int J Infect Dis 14:e102–e110
Feng JY, Huang SF, Ting WY, Chen YC, Lin YY, Huang RM, Lin CH, Hwang JJ, Lee JJ, Yu MC, Yu KW, Lee YC, Su WJ (2012) Gender differences in treatment outcomes of tuberculosis patients in Taiwan: a prospective observational study. Clin Microbiol Infect 18:E331–E337
Festenstein F, Grange JM (1999) Tuberculosis in ethnic minority groups in industrialised countries; Tuberculosis – an interdisciplinary perspective. Imperial College Press, London
Fochsen G, Deshpande K, Thorson A (2006) Power imbalance and consumerism in the doctor-patient relationship: health care providers' experiences of patient encounters in a rural district in India. Qual Health Res 16:1236–1251
Forssborhm M, Zwahlen M, Loddenkemper R, Rieder HL (2008) Demographic characteristics of patients with extrapulmonary tuberculosis in Germany. Eur Respir 31:99–105
Fox GJ, Dobler CC, Marks GB (2011) Active case finding in contacts of people with tuberculosis (Review). Cochrane Database of Systematic. The cochrane collaboration. Wiley
Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, Zeller K, Andrews J, Friedland G (2006) Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 368:1575–1580
Glaziou P, Floyd K, Korenromp EL, Sismanidis C, Bierrenbach AL, Williams BG, Atun R, Raviglione M (2011) Lives saved by tuberculosis control and prospects for achieving the 2015 global target for reducing tuberculosis mortality. Bull World Health Organ 89:573–582
Glynn JR, Crampin AC, Ngwira BM, Mwaungulu FD, Mwafulirwa DT, Floyd S, Pönnighaus JM, Warndorff DK, Fine PE (2004) Trends in tuberculosis and the influence of HIV in northern Malawi, 1988–2001. AIDS 18:1459–1463
Godfrey-Fausett P, Kaunda H et al (2002) Why do patients with a cough delay seeking care at Lusaka urban health centres? A health systems research approach. Int J Tuberc Lung Dis 6:796–805
Gounder CR, Wada NI, Kensler C et al (2011) Active tuberculosis case-finding among pregnant women presenting to antenatal clinics in Soweto, South Africa. J Acquir Immune Defic Syndr 57:e77–e84
Haas D (2000) Mycobacterium tuberculosis. Churchill Livingstone, Philadelphia
Hamid Salim MA, Declercq E, van Deun A, Saki KA (2004) Gender differences in tuberculosis: a prevalence survey done in Bangladesh. Int J Tuberc Lung Dis 8:952–957
Hoa NB, Sy DN, Nhung NV, Tiemersma EW, Borgdorff MW, Cobelens FGJ (2010) National survey of tuberculosis prevalence in Viet Nam. Bull World Health Organ 88
Holmes CB, Hausler H, Nunn P (1988) A review of sex differences in the epidemiology of tuberculosis. Int J Tuberc Lung Dis 2:96–104
Holmes CB, Hausler H, Nunn P (1998) A review of sex differences in the epidemiology of tuberculosis. Int J Tuberc Lung Dis 2:96–104
Hudelson P (1996) Gender differentials in tuberculosis: the role of socio-economic and cultural factors. Tuber Lung Dis 77
Iseman MD (2000) A clinician's guide to tuberculosis. Williams & Wilkins, Philadelphia
Jiménez-Corona ME, García-García L, DeRiemer K, Ferreyra-Reyes L, Bobadilla-del-Valle M, Cano-Arellano B, Canizales-Quintero S, Martínez-Gamboa A, Small PM, Sifuentes-Osornio J, Ponce-de-León A (2006) Gender differentials of pulmonary tuberculosis transmission and reactivation in an endemic area. Thorax 61:348–353
Johansson E, Winkvist A (2002) Trust and transparency in human encounters in tuberculosis control: lessons learned from Vietnam. Qual Health Res 12:473–491

Johansson E, Long NH, Diwan VK, Winkvist A (1999) Attitudes to compliance with tuberculosis treatment among women and men in Vietnam. Int J Tuberc Lung Dis 10:862–868
Johansson E, Long NH, Diwan VK, Winkvist A (2000) Gender and tuberculosis control: perspectives on health seeking behaviour among men and women in Vietnam. Health Policy Plan 52:33–51
Kandrack MA, Grant KR, Segall A (1991) Gender differences in health related behaviour: some unanswered questions. Soc Sci Med 32:579–590
Karim F, Islam A, Chowdhury AMR, Johansson E, Diwan VK (2007) Gender differences in delays in diagnosis and treatment of tuberculosis. Health Policy Plan 22:329–334
Khan A, Walley J, Newell J, Imdad N (2000) Tuberculosis in Pakistan: socio- cultural constraints and opportunities in treatment. Soc Sci Med 50:247–254
Khan MS, DAR O, Sismanidis C, Shah K, Godfrey-Faussett P (2007) Improvement of tuberculosis case detection and reduction of discrepancies between men and women by simple sputum-submission instructions: a pragmatic randomized controlled trial. Lancet 369
Kivihya-Ndugga LE, van Cleeff MR, Ng'ang'a LW, Meme H, Odhiambo JA, Klatser PR (2005) Sex-specific performance of routine TB diagnostic tests. Int J Tuberc Lung Dis 9:294–300
Krishnan L, Akande T, Shankar AV, Mcintire KN, Gounder CR, Gupta A, Yang WT (2014) Gender-related barriers and delays in accessing tuberculosis diagnostic and treatment services: a systematic review of qualitative studies. Tuberc Res Treat 2014:215059
Lawn SD, Zumla AI (2011) Tuberculosis. Lancet 378:57–72
Lienhardt C, Fielding K, Sillah JS, Bah B, Gustafson P, Warndorff D, Palayew M, Lisse I, Donkor S, Diallo S, Manneh K, Adegbola R, Aaby P, Bah-Sow O, Bennett S, Mcadam K (2005) Investigation of the risk factors for tuberculosis: a case-control study in three countries in West Africa. Int J Epidemiol 34:914–923
Lin CY, Chen TC, Lu PL, Lai CC, Yang YH, Lin WR, Huang PM, Chen YH (2013) Effects of gender and age on development of concurrent extrapulmonary tuberculosis in patients with pulmonary tuberculosis: a population based study. PLoS ONE 8:e63936
Long NH, Diwan VK, Winkvist A (2002) Difference in symptoms suggesting pulmonary tuberculosis among men and women. J Clin Epidemiol 55:115–120
Lonnroth K, Thuong LM, Linh PD, Diwan VK (2001) Utilization of private and public health- care providers for tuberculosis symptoms in Ho Chi Minh City, Vietnam. Health Policy Plan 16:47–54
Loto O, Awowole I (2012) Tuberculosis in pregnancy: a review. J Pregnancy 379271
Martin C, Black V (2012) Tuberculosis prevention in HIV-infected pregnant women in South Africa. South Afr J HIV Med 13
Martinez N, Rhee JT, Small PM, Behr MA (2000) Sex differences in the epidemiology of tuberculosis in San Francisco. Int J Tuberc Lung Dis 4:26–31
Mekonnen A (2014) Smear-positive pulmonary tuberculosis and AFB examination practices according to the standard checklist of WHO's tuberculosis laboratory assessment tool in three governmental hospitals, Eastern Ethiopia. BMC Res Notes 7:295
Mfinanga S, Mutayoba B, Kahwa A, Mtandu R, Kimaro G, Ngada EE (2008) The magnitude and factors responsible for delay in tuberculosis management in Dar es Salaam, Tanzania. BMC Health Serv Res 8:158
Mukherjee A, Saha I, Sarkar A, Chowdhury R (2012) Gender differences in notification rates, clinical forms and treatment outcome of tuberculosis patients under the RNTCP. Lung India 29:120–122
Munro SA, Lewin SA, Smith H, Engel ME, Fretheim A, Jimmy Volmink J (2007) Patient adherence to tuberculosis treatment: a systematic review of qualitative research. PLoS Med 4:e238
Musellim B, Erturan S, Sonmez Duman E, Ongen G (2005) Comparison of extra-pulmonary and pulmonary tuberculosis cases: factors influencing the site of reactivation. Int J Tuberc Lung Dis 9:1220–1223

Muvunyi CM, Masaisa F, Bayingana C, Musemakweri Q, Mutesa L, Hernandez TC (2010) Prevalence and diagnostics of sputum smear positive tuberculosis cases at a tertiary care institution in Rwanda. Afr J Micro Res 4:88–91

Narasimhan P, Wood J, Macintyre CR, Mathai D (2013) Risk factors for tuberculosis. Pulm Med. doi:10.1155/2013/828939

Needham DM, Foster SD, Tomlinson G, Godfrey-Fausett P (2001) Socio- economic, gender and health services factors affecting diagnostic delay for tuberculosis patients in urban Zambia. Trop Med Int Health 6:256–259

Neyrolles O, Quintanna-Murci L (2009) Sexual inequality in tuberculosis. PLoS Med 6

Ngadaya ES, Mfinanga GS, Wandwalo ER, Morkve O (2009) Pulmonary tuberculosis among women with cough attending clinics for family planning and maternal and child health in Dar Es Salaam, Tanzania. BMC Public Health 9:278

Ngamvithayapong J, Yanai H, Winkvist A, Diwan V (2001) Health seeking behaviour and diagnosis for pulmonary tuberculosis in an HIV- epidemic mountainous area of Thailand. Int J Tuberc Lung Dis 5:1013–1020

Nhamoyebonde S, Leslie A (2014) Biological differences between the sexes and susceptibility to tuberculosis. J Infect Dis 209(Suppl 3):S100–S106

Nsubuga P, Johnson JL, Okwera A, Mugerwa RD, Ellner JJ, Whalen CC (2002) Gender and HIV-associated pulmonary tuberculosis: presentation and outcome at one year after beginning antituberculosis treatment in Uganda. BMC Pulm Med 2:4

Onifade DA, Bayer AM, Montoya R et al (2010) Gender related factors in influencing tuberculosis control in shantytowns: a qualitative study. BMC Public Health 10

Onyebujoh P, Rodriguez W, Mwaba P (2006) Priorities in tuberculosis research. Lancet 367 (9514):940–942

Ottenhoff TH, Verreck FA, Hoeve MA, van de Vosse E (2005) Control of human host immunity to mycobacteria. Tuberculosis (Edinb) 85:53–64

Pronyk RM, Makhubele MB, Hargreaves JR (2001) Assessing health seeking behaviour among tuberculosis patients in rural South Africa. Int J Tuberc Lung Dis 5:619–627

Rangan S, Uplekar M (1998) Gender perspectives of access to health and tuberculosis care. Nordic School of Public Health, Göteborg

Rhines AS (2013) The role of sex differences in the prevalence and transmission of tuberculosis. Tuberculosis (Edinb) 93:104–107

Rieder HL (1999) Epidemiological basis of tuberculosis control. International Union Against Tuberculosis and Lung Disease, Paris

Sanchez-Perez HJ, Hernan MA, Hernandez-Diaz S et al (2002) Detection of pulmonary tuberculosis in Chiapas. Ann Epidemiol 12:166–172

Seddon JA, Hesseling AC, Godfrey-Faussett P, Fielding K, Schaaf HS (2013) Risk factors for infection and disease in child contacts of multidrug-resistant tuberculosis: a cross-sectional study. BMC Infect Dis 13:392

Sreeramareddy C, Panduru KV, Verma SC (2008) Comparison of pulmonary and extra pulmonary tuberculosis in Nepal-A hospital based retrospective study. BMC Infect Dis 8

Thorson A (2003) Equity and equality – case detection of tuberculosis among women and men in Vietnam. Ph.D. thesis, Karolinska Institute University Press

Thorson A, Diwan VK (2001) Gender inequalities in tuberculosis: aspects of infection, notification rates, and compliance. Curr Opin Pulm Med 7:165–169

Thorson A, Johansson E (2004) Equality or equity in health care access: a qualitative study of doctors' explanations to a longer doctor's delay among female TB patients in Vietnam. Health Policy 68:37–46

Thorson A, Hoa NP, Long NH (2000) Health- seeking behaviour of individuals with a cough of more than 3 weeks. Lancet 356:1823–1824

Thorson A, Hoa NP, Long NH et al (2004) Do women with tuberculosis have a lower likelihood of getting diagnosed? Prevalence and case detection of sputum smear positive pulmonary TB, a population based study from Vietnam. J Clin Epidemiol 57:398–402

Thorson A, Long NH, Larsson LO (2007) Chest X- ray findings in relation to gender and symptoms: a study of patients with smear positive tuberculosis in Vietnam. Scand J Infect Dis 39:33–37

Tsankova G, Kaludova V, Georgieva E, Ermenlieva N (2014) Gender disparity in prevalence of tuberculosis in region Varna in 2012. J IMAB - Annu Proc (Scientific Papers) 20:498–499

Tulu B, Dida N, Kassa Y, Taye B (2014) Smear positive pulmonary tuberculosis and its risk factors among tuberculosis suspect in South East Ethiopia; a hospital based cross-sectional study. BMC Res Notes 7:285

UNAIDS (2007) AIDS epidemic update. http://www.unaids.org

Uplekar MW, Rangan S, Weiss MG, Ogden J, Borgdorff MW, Hudelson P (2001) Attention to gender issues in tuberculosis control. Int J Tuberc Lung Dis 5:220–224

van Lunzen J, Altfeld M (2014) Sex differences in infectious diseases-common but neglected. J Infect Dis 209(Suppl 3):S79–S80

Verbrugge LM (1989) The twain meet: empirical explanations of sex differences in health and mortality. J Health Soc Behav 30:282–304

Vlassoff C, Bonilla E (1994) Gender- related differences in the impact of tropical diseases on women: what do we know? J Biosoc Sci 26:37–53

Walzl G, Beyers N, Helden P (2005) TB: a partnership for the benefit of research and community. Transac R Soc Trop Med Hygiene 99(Suppl 1): S15–S19

Wang W, Jiang Q, Saleh A, ABDULLA M (2007) Barriers in accessing tuberculosis care among non-residents in Shanghai: a descriptive study of delays in diagnosis. Eur J Public Health 17:419–423

Watkins RE, Plant AJ (2006a) Does smoking explain sex differences in the global tuberculosis epidemic? Epidemiol Infect 2006(134):333–339

Watkins RE, Plant AJ (2006b) Does smoking explain sex differences in the global tuberculosis epidemic? Epidemiol Infect 134:333–339

WHO (2006) Gender and tuberculosis: cross- site analysis and implications of a multi- country study in Bangladesh, India, Malawi and Colombia. Report series no. 3, Special Programme for Research and Training in Tropical Diseases (TDR). World Health Organization, Geneva

WHO (2012) World Health Organization (WHO). Tuberculosis country profiles: South Africa. Geneva. http://www.who.int/tb/data

WHO (2013) World Health Organization: global tuberculosis report

Wood R, Racow K, Bekker LG et al (2012) Indoor social networks in a South African township: potential contribution of location to tuberculosis transmission. PLoS ONE 7

Yamasaki-Nakagawa M, Ozasa K, Yamada N et al (2001) Gender difference in delays to diagnosis and health care seeking behaviour in a rural area of Nepal. Int J Tuberc Lung Dis 5:24–31

Chapter 9
Sex Differences in Sepsis Following Trauma and Injury

Huang-Ping Yu and Irshad H. Chaudry

Abstract The sex of a patient is increasingly recognized as a major factor determining the outcome of sepsis. Experimental findings indicate that female rodents in the proestrus cycle (i.e., when the estrogen levels are at their highest) are more tolerant than their male counterparts to major injuries. Several lines of evidence indicate that male and female humans and rodents respond differently to shock. In this regard, findings from clinical studies suggest that premenopausal women have a lower incidence of infection, pneumonia, sepsis, and multiple organ failure than men under those conditions. Sex differences have also been noted in organ function, and the potential reasons for these differences have been the subject of extensive research. This chapter deals with sepsis following trauma and injury and examines the following: (1) the evidence for sex differences following trauma and sepsis and (2) the mechanisms by which gender/sex hormones provide organ protection under those conditions. The available information indicates that sex steroids modulate organ function following injury. Thus, modulation of the prevailing hormone milieu immediately following injury appears to be a novel therapeutic approach for improving the outcome following those conditions.

9.1 Sepsis in Humans

Severe sepsis is known to produce many life-threatening sequelae. Numerous advances in the past have improved short-term survival of patients in intensive care units; however, despite these advances, sepsis and multiple organ failure still remain the leading causes of morbidity and mortality in severely injured patients who survive the initial trauma (Angele et al. 2000; Baue 2000; Bruhn et al. 2006;

H.-P. Yu
Department of Anesthesiology, Chang Gung Memorial Hospital, Taoyuan Hsien, Taiwan ROC

I.H. Chaudry (✉)
Center for Surgical Research and Department of Surgery, University of Alabama at Birmingham, G094 Volker Hall, 1670 University Boulevard, Birmingham, AL 35294-0019, USA
e-mail: Ichaudry@uab.edu

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_9

Choudhry et al. 2005; Cotton et al. 2006; Finnerty et al. 2007; Gauglitz et al. 2008; Holcomb et al. 2007; Hsieh et al. 2005, 2006a; Kamoun et al. 2005; Kawasaki et al. 2008; Kher et al. 2005; Kobbe et al. 2008; MacConmara et al. 2006; Moore et al. 2005; Murakami et al. 2007; Purcell et al. 2006; Remick and Ward 2005; Rossaint et al. 2006; Tsujimoto et al. 2008; Wheeler et al. 2008; Yu et al. 2006a, b, c, d, e, f).

Sepsis is a common complication of traumatic injury, developing in up to one-half of all trauma patients (Angus et al. 2001; Baue et al. 1998; Vincent 2000). Furthermore, the early, acute events of sepsis may trigger long-term consequences such as lingering immunosuppression and pulmonary complications (Benjamim et al. 2004; Reddy et al. 2001) and can lead to late mortality in a quarter of survivors of severe sepsis (Benjamim et al. 2004). Prevention of sepsis and septic complications has received much attention. Despite a substantial expenditure of effort and resources, little headway has been made in reducing the frequency of septic complications and trauma-associated mortality and morbidity in patients (Deitch 1998; Esmon 2004).

9.2 Experimental Model of Sepsis

The purpose of using a reproducible animal model of sepsis is to have a controlled setting that decreases the number of variables so that one can study in detail the mechanisms responsible for the altered immunological, cardiovascular, and metabolic changes under those conditions. Only by thoroughly understanding the mechanism of the pathophysiology of sepsis and septic shock can we devise better and effective therapeutic modalities (Wichterman et al. 1980). Freise et al. (2001) make the point that models that successfully replicate the conditions of clinical sepsis tend to focus on infection rather than on systemic initiation. In addition, they point out that a septic focus allows for further evaluation of treatments such as antibiotic therapy, volume resuscitation, and surgery. The cecal ligation and puncture (CLP) model for sepsis (Wichterman et al. 1980; Hubbard et al. 2005) may replicate the nature and course of clinical sepsis in patients after trauma. Other putative models of sepsis include intravenous infusion of endotoxin or live organisms, the administration of fecal material or live organisms into the peritoneal cavity, the placement of infected foreign material into the soft tissue of the extremities to produce abscesses, and surgical operations to partially destroy the normal barrier of the gastrointestinal tract to simulate the conditions that occur, for instance, after bowel infarction or cholecystitis (Wichterman et al. 1980; Hubbard et al. 2005).

9.3 Sex Differential Response and Outcome After Injury in Humans

Clinical studies have shown that women in the childbearing age have a lower incidence of pneumonia, sepsis, and multiple organ failure than men after trauma (Gannon et al. 2004; Mostafa et al. 2002; Offner et al. 1999). In a study by Frink et al. (2007a) involving polytraumatized patients, women showed lower interleukin (IL)-6, and IL-8 levels, which were associated with less multiple organ dysfunction syndrome and sepsis. In another study, Wichmann et al. (2000) showed that adult females developed sepsis less frequently than males during the clinical course in surgical intensive care units, although once sepsis developed, the mortality rate was the same for men and women. Consistent with these findings, Offner et al. (1999) reported that being male is associated with an increased risk of major infection following trauma. Some studies reported a benefit only in women >50 years of age (George et al. 2003a), whereas others showed a benefit only in women <50 years of age (Mostafa et al. 2002; Wohltmann et al. 2001). On the other hand, although males have been reported to have a higher incidence of pneumonia, which is described as the most common infection following severe trauma (Gannon et al. 2004), an established diagnosis of pneumonia was associated with higher mortality in females (Napolitano et al. 2001). In another study, being female predicted increased mortality in critically ill surgical patients with documented infection and after certain elective or emergency surgical procedures (Eachempati et al. 1999). Some investigators suggest that there is no difference between the sexes in mortality after trauma (Gannon et al. 2002; Napolitano et al. 2001), while other studies demonstrated a higher mortality rate in women >80 years of age compared with age-matched males (Eachempati et al. 1999). A recent study by Deitch et al. involving over 4,000 patients showed that regularly cycling women tolerate shock-trauma better than age-matched men (Deitch et al. 2007). Even though most clinical studies demonstrated an advantage for females over males, a definitive answer regarding the role of sex in the outcome following trauma and sepsis remains controversial. The reasons for the differences in results are several and include lack of knowledge of the hormonal status of the patient at the time of injury.

Croce et al. (2002) found no sex difference in severity of penetrating or blunt trauma patients. In addition, they separated blunt trauma patients into those ≤40 years or >50 years of age and found no sex-related mortality difference. Following a similar analysis, the data from George et al. (2003b) showed a statistically significant sex difference in the ≤50 age group, with male patients having a 2.5 times higher risk of death than females following trauma.

Another reason for the discrepant results may be that the hormonal status of the patients was not accounted for, and thus, females were likely in different phases of the menstrual cycle [i.e., with different circulating 17β-estradiol (E2) levels] at the time of injury. This may explain why similar findings are not observed in different studies. More precise patient grouping by cycle status would allow a clearer

understanding of the hormone-related relationship to disease outcome. Future clinical trials should partition and analyze data with respect to oral contraception, hormone replacement therapy, or surgical history. Knowledge of menstrual cycle stage as well as exogenous hormone use may provide a clearer understanding of the association between hormone patterns and the outcome from trauma. Thus, more clinical studies that include hormone measurement at the time of injury are needed to understand the role of sex and sex steroids in post-trauma pathogenesis.

9.4 Sex Difference in Animal Models of Sepsis/Trauma

In contrast to findings in humans, animal studies have consistently found advantages for proestrus females under stressful conditions. Ovariectomized female rats have worse cardiac functional recovery following trauma-hemorrhage (T-H) than intact control animals (Jarrar et al. 2000a). Their recovery is markedly improved by the administration of 17β-estradiol (E2) (Jarrar et al. 2000a). Furthermore, female rats in proestrus cycle (i.e., the stage of the estrous cycle in which E2 levels are the highest) show normal cardiac and pulmonary function following T-H and resuscitation (Schneider et al. 2000; Yu et al. 2006a). In contrast, female rats in diestrus (i.e., the stage of the estrous cycle when E2 levels are lowest) have depressed cardiac and hepatic function that is similar to males following T-H (Jarrar et al. 2000b). The beneficial effects of E2 are also evident in male rats treated with E2 as a therapeutic modality following T-H induction (Angele et al. 1999, 2000; Hsieh et al. 2005). Moreover, E2 maintains cardiovascular and liver functions under stress conditions (e.g., following T-H) (Hsieh et al. 2005; Szalay et al. 2006). Studies have also shown that administration of progesterone following T-H in sex steroid-deficient female rats improved cardiovascular functions (Kuebler et al. 2003). A pivotal role for androgens has been suggested in producing the depression in organ functions following T-H, because both castration (Angele et al. 1999) and androgen receptor antagonism (Hsieh et al. 2006a; Wichmann et al. 1997) improve/restore organ functions following T-H (Angele et al. 2000).

The rodent model of trauma and hemorrhage provides a basis for experimental investigation of the immune and physiologic responses to a controlled insult. However, the model may more closely resemble a clinical situation of penetrating rather than blunt trauma because there is a lack of diffuse tissue injury. Accordingly, it must be pointed out that the model yields only soft tissue injury without direct organ injury. Although exceedingly useful for the study of a soft tissue injury response (i.e., without internal organ damage) and potential therapeutic interventions, the animal model utilized may not adequately represent the clinical picture of either blunt or complex penetrating trauma. Similarly, differences in trauma patients must be considered: blunt- and penetrating-injured patients may vary with respect to comorbidities, injury patterns, and treatment protocols. Therefore, a clear definition of the patient population to which laboratory findings can be

applied is necessary in order to translate the laboratory findings into the clinical arena in both a safe and efficient manner.

During menopause, luteinizing hormone and follicle-stimulating hormone levels increase to those well above premenopausal levels; E2 and estrone levels decrease; and to a lesser extent, as a function of age, androstenedione and testosterone levels decline, characterizing adrenopause in men (Lobo 2000). In addition, human postmenopausal prolactin pulsatile frequencies and levels resemble those of males rather than those of premenopausal females (Katznelson et al. 1998). Therefore, the beneficial effects of prolactin described by Zellweger et al. (1996) may contribute to the mortality differences observed in blunt trauma patients, and the presumed E2 effect may be a marker for premenopausal prolactin levels and pulsatile frequencies. Unfortunately, a clinical determination of the importance of prolactin is complicated by the pulsatile frequency and diurnal variations, highlighting the importance of laboratory investigations.

It should be noted that similarities and differences exist between the clinical and laboratory data, suggesting that the patient hormonal milieu may affect outcome. Additional research is needed to clarify the patient subpopulations for which the sex difference is most evident; however, prospective studies in which cycle status, medical history, and surgical history are recorded will provide the basis for potential intervention should the hormonal status impart a clinically relevant effect on outcome. Alternatively, it may be easier to administer E2 to patients in the emergency room and determine if the incidence of organ dysfunction, complications, and length of hospital stay decreases when E2 is used as an adjuvant to traditional treatments.

9.5 Sex Hormone Receptors After Trauma

The functions of vital organs such as heart, liver, lung, and intestine are compromised following T-H in males but not in proestrus females. Studies have shown that cardiac function, as determined by cardiac output, stroke volume, contractility, and total peripheral resistance, is markedly depressed after T-H in males and females in estrus, metestrus, or diestrus phase, as well as in ovariectomized females, despite fluid resuscitation (Choudhry et al. 2005; Yang et al. 2006). In contrast, cardiac function is maintained in proestrus females following T-H (Choudhry et al. 2005; Yang et al. 2006). Furthermore, administration of E2 in either male or ovariectomized female rats protects cardiac function following T-H (Choudhry et al. 2005). Similarly, T-H produces lung, liver, and intestinal tissue edema within a few hours after injury, and like cardiac function, tissue edema is not observed in proestrus female or in male rats treated with E2 following T-H (Frink et al. 2007b; Yu et al. 2006c). Additional findings indicate that an increase in lung myeloperoxidase (MPO) activity, neutrophil chemokines [e.g., cytokine-induced neutrophil chemoattractant 1 (CINC-1), CINC-3], and intercellular adhesion molecule-1 expression is elevated in females during diestrus and estrus as well as

in ovariectomized female rats (Yu et al. 2006a). The highest level of E2 in proestrus females was associated with the lack of lung inflammation following T-H, whereas all other stages of the estrus cycle had significantly lower plasma E2 levels and significant lung inflammation following T-H (Choudhry et al. 2005; Yang et al. 2006; Yu et al. 2006a). Although E2 levels were relatively higher in female rats in estrus and metestrus cycles compared to the levels seen in ovariectomized females, the finding of increased lung injury markers in those animals suggests that E2 levels in the estrus and metestrus cycles were not sufficient to attenuate lung injury following T-H. Thus, sex steroids are, at least in part, responsible for improving/maintaining organ functions following T-H. Estrogen appears to be causative factor in the maintenance of organ function both in males and females after T-H, and its administration in male rats and ovariectomized female rats following T-H helps to prevent organ dysfunction under those conditions. Since administration of a single dose of E2 following T-H produced salutary effects, it is unlikely that such a dose E2 produces any potential ill effects of E2 that are produced when E2 is used for long periods of time such as during hormone replacement therapy. Thus, the beneficial effects of administration of a single dose of E2 following T-H would not be expected to be associated with the deleterious consequences of long-term E2 treatment such as increased risk of breast cancer, endometrial cancer, and coagulation (Gruber et al. 2002).

Sex hormone-mediated effects are primarily mediated by their receptors. Coadministration of E2 with ICI 182,780, a selective ER antagonist, abolished the salutary effects of E2 on cardiac function following T-H in rats (Jarrar et al. 2000a). Moreover, progesterone-mediated cardioprotection is associated with increased progesterone receptor activity in the left ventricle and increased circulating blood volume following T-H (Kuebler et al. 2003). Administration of flutamide, an androgen receptor antagonist, restores cardiac and hepatic functions and decreased intestinal neutrophil infiltration after T-H in male rats (Hsieh et al. 2005, 2006a; Yu et al. 2006b). Flutamide administration following T-H also increases cardiac E2 levels and ER expression through upregulation of aromatase activity, which converts testosterone to E2 (Hsieh et al. 2006a).

9.5.1 ER-α Reduced Inflammatory Response in Liver and Small Intestine and ER-β Attenuated Inflammatory Response in Lung and Small Intestine

Action of E2 is mainly mediated by two intracellular ERs designated ER-α and ER-β (Hsieh et al. 2006b, c). Previous studies have shown tissue-specific expression of subtypes of ERs (Kuiper et al. 1997). For example, rat liver was found to be rich in ER-α and lung in ER-β (Yu et al. 2006c). Alternatively, intestine is rich in both ER-α and ER-β. Recent studies have shown the role of ERs in E2-mediated protection of various organ functions following T-H in rats. These studies used

ER-α- and ER-β-specific agonists, propylpyrazole triol (PPT) and diarylpropionitrile (DPN), respectively. PPT is a selective agonist for the ER-α subtype and is the most potent agonist for ER-α of a series of tetrasubstituted pyrazole analogs (Yang et al. 2006). PPT binds to ER-α with high affinity, displaying 410-fold binding selectivity over ER-β (Yang et al. 2006). DPN, on the other hand, acts as an agonist on both intracellular ER subtypes but has a 70-fold higher relative binding affinity and 170-fold higher relative estrogenic potency in transcription assays with ER-β than ER-α (Frink et al. 2007b). The liver, small intestine, and lung are considered critical organs in the development of delayed organ dysfunction in patients suffering from traumatic injuries and severe blood loss. Multiple organ dysfunction or failure secondary to a systemic inflammatory response remains the major cause of morbidity and mortality following trauma (Wu et al. 2001). Neutrophils are the principal cells involved in host defense against acute bacterial and fungal infections (Malech and Gallin 1987), and thus, these cells have a protective effect. However, under shock conditions, the infiltration of these cells may cause tissue damage (Angle et al. 1998). Neutrophil tissue migration is mediated by multiple adhesion molecules on the neutrophil and endothelial cell surfaces as well as chemotactic factors. Among adhesion molecules, ICAM-1 is an important mediator of the adhesion of neutrophils to the vascular endothelium and is markedly upregulated following T-H in rats (Dayal et al. 2002).

With regard to chemokines, rat CINC-1 and CINC-3 are members of the IL-8 family and are potent chemotactic factors for neutrophils. Recruitment and chemotaxis of neutrophils into tissue is a key event in inflammation. Using CINC antibodies, studies have shown that CINC-1 and CINC-3 contribute significantly to the influx of neutrophils in rat inflammation models including lung injury (Shanley et al. 1997) and lipopolysaccharide-induced inflammation (Iida et al. 1992). Additional studies indicate that CINC-1 levels correlated with tissue MPO activity, a marker of neutrophil content, following T-H (Yu et al. 2006a; Yu et al. 2006c). Studies have also indicated that E2-induced reduction of MPO activity (an index of neutrophil infiltration), chemoattractants CINC-1 and CINC-3, and intercellular adhesion molecule-1 following T-H are mediated via ER-α activation in the liver, via ER-β activation in the lung, and via both ER-α and ER-β in the small intestine (Yu et al. 2006c). These findings are consistent with ER mRNA expression in the liver, small intestine, and lung (i.e., ER-α mRNA expression is highest in the liver and ER-β mRNA expression is greatest in the lung) (Yu et al. 2006c). Thus, such differences in distribution of ER subtypes in various tissues contribute to the selective role of ER-α or ER-β in response to tissue injury (Kuiper et al. 1997). Furthermore, administration of the ER-α agonist PPT attenuates hepatic injury and decreases the expression of the nuclear factor-kappaB (NF-κB), activating protein 1 (AP-1) and inducible nitric oxide synthase (iNOS) in the liver following T-H (Shimizu et al. 2007). The iNOS is significantly upregulated in the liver after hemorrhagic shock and is thought to be one of the major contributors of hepatic injury following hemorrhagic shock or sepsis (Menezes et al. 2002). Furthermore, a positive correlation between hepatic injury and increased iNOS expression has been

shown in rats (Shimizu et al. 2007). The production of proinflammatory mediators is regulated by NF-κB and AP-1 (Meldrum et al. 1997). A recent study has demonstrated cross talk between ERs and NF-κB at several levels (Kalaitzidis and Gilmore 2005), for example, the inhibition of NF-κB target genes by ER-α and ER-α inhibition of NF-κB-mediated transcriptional induction of IL-6 gene. ERs also activate transcription at alternative sites, such as AP-1 (Kushner et al. 2000). Furthermore, administration of DPN in male rats attenuated T-H-mediated increase in protein concentration, LDH activity, nitrate/nitrite, and IL-6 levels in bronchoalveolar fluid (Yu et al. 2006d). It also decreased iNOS expression, nitrate/nitrite, and IL-6 levels in the lung (Yu et al. 2006d). In addition, it appears that the salutary effects of E2 on attenuation of iNOS expression and NO production in the lung are receptor dependent. Support for this suggestion comes from the study which showed that administration of E2 with ER antagonist ICI 182,780 abolished the salutary effects of E2 in the lung in rats (Cuzzocrea et al. 2000). Another study provides further evidence that following T-H, E2-induced attenuation of lung injury is mediated via ER-β activation in male rats (Yu et al. 2006d).

iNOS can be overexpressed in rodent lungs following hemorrhagic shock (Kiang et al. 2005). Hierholzer and colleagues have reported that iNOS inhibition results in a marked reduction of lung injury produced by hemorrhagic shock (Hierholzer et al. 1998). These findings support the view that an enhanced formation of NO from iNOS plays an important role in producing lung injury following hemorrhagic shock (Hierholzer et al. 1998), which can be altered by E2 signaling through ERs.

9.5.2 ER-β-Mediated Cardiac Protection by Upregulation of Heat-Shock Proteins

Studies have also shown that DPN improve cardiac function and increase heat-shock proteins (HSPs) 32, 60, 70, and 90 and heat-shock factor-1 (HSF-1) DNA-binding activity in the heart following T-H in male rats (Yu et al. 2006e). E2 has been reported to provide protection against vascular injury even in female mice in which ER-α has been disrupted (Iafrati et al. 1997). Moreover, the expression of ER-β, but not of ER-α, is stimulated after vascular injury in male rats (Lindner et al. 1998). Furthermore, studies utilizing ER-α or ER-β knockout mice suggest that ER-β plays a role in cardioprotection following ischemia-reperfusion (Gabel et al. 2005). The HSPs are an important family of endogenous, protective proteins. HSP70 is induced by brief ischemia, and overexpression of HSP70 protects cells and tissues against various forms of stress, including heatstroke-induced circulatory shock and cerebral ischemia (Wang et al. 2005). Conversely, reduced expression of HSP70 resulting from treatment with antisense oligonucleotides to HSP70 increases susceptibility to hypoxia and reoxygenation injury in adult feline cardiocytes (Nakano et al. 1997). Overexpression of other HSPs including HSP32 and HSP60 is also reported to be protective against cardiac injury in rats (Szalay

et al. 2005). HSP synthesis is controlled by a family of transcription factors, the HSFs. Four HSFs have been identified in rats, but only HSF-1 has been shown to regulate the expression of HSPs in response to ischemia, hypoxia, heat, stretch, or injury (Nishizawa et al. 2002). Heat and hypoxia activate HSF-1, which is present in the cytoplasm in an inactive, monomeric form. With stress, trimerization as well as phosphorylation occurs following which HSF-1 migrates to the nucleus. In the nucleus, HSF-1 binds to the heat-shock element, which is present in the promoter of the stress response gene, and then initiates HSP transcription and synthesis. HSP90 is known to bind to intracellular steroid receptor, including the ERs (Knowlton and Sun 2001). HSP90 might also complex with HSF-1 in cardiomyocytes (Knowlton and Sun 2001). Interactions involving HSP90 and ERs, as well as the binding between HSP90 and HSFs, represent an important element in the activation of HSF-1 by E2 (Knowlton and Sun 2001). Several studies have examined the effects of E2 and sex on cardiac HSP expression in rodents (Knowlton and Sun 2001; Voss et al. 2003). These studies have shown that female rat hearts have twice as much HSP70 as hearts from males (Voss et al. 2003). Ovariectomy of female rats reduced the level of HSP70 in the heart, which can be reversed by E2 administration (Voss et al. 2003). Additional studies show that 10 h of E2 treatment doubled the level of HSP70 in adult cardiomyocytes from male rats (Knowlton and Sun 2001). Consistent with these findings, treatment with E2 increases the expression of HSP32 in the rat heart following T-H (Szalay et al. 2005). Upregulation of HSP synthesis is a powerful physiological, endogenous route for protecting crucial cellular homeostatic mechanisms against deleterious external factors. Physiological stresses ranging from myocardial ischemia to genetic mutations produce a disease state in which protein damage and misfolded protein structures are a common denominator (Kumarapeli and Wang 2004). Multiple endogenous pathways are involved in restoring cellular homeostasis, but one well-characterized mechanism that involves protein folding is the heat-shock family of stress proteins, i.e., HSPs (Benjamin and McMillan 1998; Menezes et al. 2002). There are several potential mechanisms by which HSPs produce cardioprotective effects. HSPs are generally thought to be useful in correcting the folding of many proteins and restore their functional structures (Benjamin and McMillan 1998). Moreover, HSPs target denatured proteins to the lysosome for degradation as molecular chaperones (Benjamin and McMillan 1998). These functions of HSPs as molecular chaperones play important roles in maintaining the normal cell functions and promoting cell survival. HSPs are also known to regulate the process of programmed cell death/apoptosis. One major pathway of apoptosis involves the release of cytochrome C from mitochondria. Cytochrome C, in turn, binds to a protein known as apoptotic protease activating factor1 (Apaf1) and triggers its oligomerization. This complex then attracts the inactive unprocessed pro-form of the proteolytic enzyme caspase-9 which is then cleaved to its active form, thereby initiating apoptosis. HSPs have been shown to inhibit this process at various points. HSP90 binds to Apaf1 and prevents it binding to cytochrome C (Pandey et al. 2000). Furthermore, HSP70 prevents oilgomerized Apaf1 from recruiting pro-caspase-9 (Beere et al. 2000). Studies have also suggested an antiapoptotic role of HSP60 (Kirchhoff et al. 2002; Lin et al.

2001). Overexpression of HSP60 inhibits myocardial apoptosis in response to ischemic injury in rat neonatal cardiac myocytes (Lin et al. 2001). Furthermore, a recent study has shown that reducing HSP60 expression with antisense oligonucleotides is associated with an increase in Bax and a reduction in Bcl-2, which induces apoptosis of cardiomyocytes (Kirchhoff et al. 2002). These findings raise the possibility that HSP60 may regulate apoptosis through modulation of the Bcl-2 family (Kirchhoff et al. 2002). In addition, HSP90 has been shown to bind to endothelial NOS (eNOS) and stimulate its activity (Shi et al. 2005). Thus, the HSPs protect cells via multiple mechanisms which target key cellular components and regulatory processes. A previous study suggests that E2-mediated restoration of cardiac function following T-H in rats is due in part to ER-dependent upregulation of PGC-1α (Hsieh et al. 2006a). The nuclear coactivator PGC-1α, known for its role in cellular metabolism, regulates a number of genes required for lipid metabolism and ATP production by activating transcription factor PPAR-α and mitochondrial transcription factor A (Tfam), respectively (Hsieh et al. 2005). It is well known that lipids produce ATP through mitochondrial fatty acid β-oxidation. The PPAR-α regulates genes involved in lipid transport and mitochondrial fatty acid β oxidation, including FAT/CD36, and medium chain acyl-coenzyme A dehydrogenase (MCAD) (Erol et al. 2004). The mitochondrial transcription factor, Tfam, transactivates mitochondrial DNA-encoded gene cytochrome *c* oxidase subunit I that is required for mitochondrial ATP production (Hsieh et al. 2005). Studies have shown that DPN treatment also attenuated the decrease in cardiac mitochondrial ATP, abrogated the T-H-induced lipid accumulation, and normalized peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α, mitochondrial transcription factor A, and cytochrome c oxidase subunit I after T-H in rats (Hsieh et al. 2006b). Likewise, it has been reported that PGC-1α expression can be induced by transcription factor CREB (cyclic-AMP response element binding). PGC-1α mRNA levels were reduced in the CREB knockout mice and sequence analysis of the mouse PGC-1α promoter reveals a full consensus CREB binding site (Herzig et al. 2001). Studies have also shown that E2 increased the enhancer activity of CREB binding and CREB protein levels (Kanda and Watanabe 2004). Moreover, the effects of E2 are through both ER-α and ER-β to increase CREB phosphorylation (Wade and Dorsa 2003). Furthermore, ER antagonist ICI 182,780 blocks the increase in CREB phosphorylation induced by E2 in a hippocampal cell line (Wade and Dorsa 2003). However, it remains to be determined whether ER-β-mediated PGC-1α upregulation following T-H is through CREB phosphorylation. Furthermore, it has long been thought that sex hormone receptors, including ERs, are localized in the cytoplasm and nucleus of the cell. However, there is evidence indicating that ERs are also localized in mitochondria, which might enhance the level of mitochondrial DNA (mtDNA)-encoded transcription directly (Chen et al. 2004). A recent study has shown that E2 enhances the mitochondrial level of ERs and increases the transcriptional levels of several mtDNA-encoded genes required for mitochondrial respiratory complex (MRC) proteins and MRC activity (Chen et al. 2004). These observations suggest that mtDNA-encoded MRC could be a direct target for E2 action in the mitochondrial ERs. Studies have also examined

the role of mitochondria in E2-mediated cardioprotection following T-H (Hsieh et al. 2006c). Male rats received PPT, DPN, or E2 following T-H, and the effects of these treatments were examined on mtER-α, mtER-β, mitochondrial estrogen response element-binding activity, and mtDNA-encoded genes for MRC-I and MRC-IV proteins (Hsieh et al. 2006c). To determine the role of MRC-IV in DPN-mediated cardioprotection, a group of DPN-treated rats was co-treated with MRC-IV inhibitor sodium cyanide. The results showed that DPN or E2 treatment after T-H normalized cardiac mtER-β expression and increased mtER-β DNA-binding activity. This was accompanied by an increase in MRC-IV gene expression and activity; MRC-I gene expression remained unchanged. Inhibition of MRC-IV in DPN-treated T-H rats by sodium cyanide abolished the DPN-mediated cardioprotection, ATP production, mitochondrial cytochrome c release, caspase-3 cleavage, and apoptosis (Hsieh et al. 2006c). Thus, E2- and ER-β-mediated cardioprotection following T-H appears, at least in part, to be mediated via mtER-β-dependent MRC-IV activity and inhibition of mitochondrial apoptotic signaling pathways.

9.6 Conclusion

A number of studies indicate that cardiovascular and immunological functions are markedly depressed following T-H in young male rodents, but they are not depressed in proestrus female rodents under those conditions. Furthermore, young male rodents are extremely susceptible to sepsis following T-H, whereas proestrus females are resistant to sepsis under those conditions. Thus, the hormonal milieu at the time of injury dictates whether the host will be immunologically depressed and susceptible to sepsis or they will be tolerant to sepsis under those conditions.

There is increasing evidence that sex hormones can have beneficial effects on organ function following injury and sepsis in humans and experimental animals. Studies have examined the role of E2 in post-shock pathogenesis (Fig. 9.1). The findings indicate that trauma/sepsis impairs organ functions. Administration of E2 to either male or female rodents following injury attenuates organ injury. Although clinical studies indicate that premenopausal females tolerate trauma and sepsis better than age-matched males, there are some clinical studies which do not support that contention. The reason for this discrepancy of results could be due to the fact that the hormonal status of the females is usually not measured immediately after injury and the cycle stops after that condition, and thus females may be in the different phases of the estrus cycle at the time of injury. Determining the hormonal status immediately following injury will therefore provide better information about the role of hormones on tolerance and susceptibility to trauma and sepsis.

The findings also indicate that there are two major receptors, ER-α and ER-β, which mediate E2 actions during T-H. Studies have shown that tissue-specific expression of subtypes of ER mediate the protective effects of E2 on organ function following trauma in rodents. Thus, alteration or modulation of the prevailing

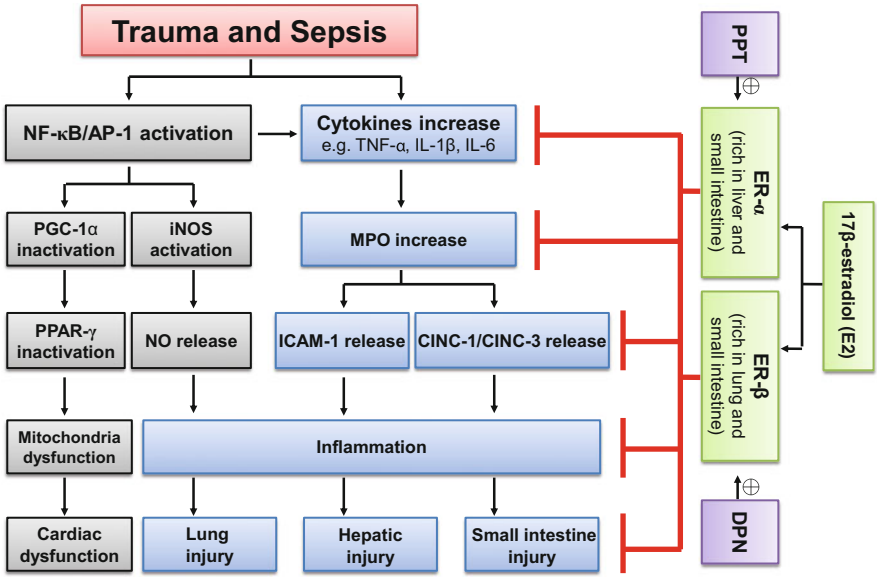

Fig. 9.1 Schematic illustration of the protective effect of estrogen following trauma and sepsis. ER-α, estrogen receptor-α; ER-β, estrogen receptor β; PPT, propylpyrazole triol; DPN, diarylpropionitrile; NF-κB, nuclear factor—kappa B; AP-1, activator protein-1; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; PPAR-γ, peroxisome proliferator-activated receptor-γ; iNOS, inducible nitric oxide synthase; NO, nitric oxide; MPO, myeloperoxidase; ICAM-1, intercellular adhesion molecule-1; CINC-1, cytokine-induced neutrophil chemoattractant-1; CINC-3, cytokine-induced neutrophil chemoattractant-3

hormonal milieu at the time of injury appears to be a novel therapeutic option for improving organ function under those conditions. However, this complex network needs additional elucidation in future experimental studies and clinical trials so that more effective therapies can be formulated.

References

Angele MK, Knoferl MW, Schwacha MG, Ayala A, Cioffi WG, Bland KI, Chaudry IH (1999) Sex steroids regulate pro- and anti-inflammatory cytokine release by macrophages after trauma hemorrhage. Am J Physiol 277:C35–C42

Angele MK, Schwacha MG, Ayala A, Chaudry IH (2000) Effect of gender and sex hormones on immune responses following shock. Shock 14:81–90

Angle N, Hoyt DB, Coimbra R, Liu F, Herdon-Remelius C, Loomis W, Junger WG (1998) Hypertonic saline resuscitation diminishes lung injury by suppressing neutrophil activation after hemorrhagic shock. Shock 9:164–170

Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR (2001) Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 29:1303–1310

Baue AE (2000) A debate on the subject "Are SIRS and MODS important entities in the clinical evaluation of patients?" The con position. Shock 14:590–593

Baue AE, Durham R, Faist E (1998) Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): are we winning the battle? Shock 10:79–89

Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T, Tailor P, Morimoto RI, Cohen GM, Green DR (2000) Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. Nat Cell Biol 2:469–475

Benjamim CF, Hogaboam CM, Kunkel SL (2004) The chronic consequences of severe sepsis. J Leukoc Biol 75:408–412

Benjamin IJ, McMillan DR (1998) Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. Circ Res 83:117–132

Bruhn A, Verdant C, Vercruysse V, Su F, Vray B, Vincent JL (2006) Effects of dexamethasone on macrophage migration inhibitory factor production in sepsis. Shock 26:169–173

Chen JQ, Eshete M, Alworth WL, Yager JD (2004) Binding of MCF-7 cell mitochondrial proteins and recombinant human estrogen receptors alpha and beta to human mitochondrial DNA estrogen response elements. J Cell Biochem 93:358–373

Choudhry MA, Schwacha MG, Hubbard WJ, Kerby JD, Rue LW, Bland KI, Chaudry IH (2005) Gender differences in acute response to trauma-hemorrhage. Shock 24(Suppl 1):101–106

Cotton BA, Guy JS, Morris JA Jr, Abumrad NN (2006) The cellular, metabolic, and systemic consequences of aggressive fluid resuscitation strategies. Shock 26:115–121

Croce MA, Fabian TC, Malhotra AK, Bee TK, Miller PR (2002) Does gender difference influence outcome? J Trauma 53:889–894

Cuzzocrea S, Santagati S, Sautebin L, Mazzon E, Calabro G, Serraino I, Caputi AP, Maggi A (2000) 17 beta-estradiol antiinflammatory activity in carrageenan-induced pleurisy. Endocrinology 141:1455–1463

Dayal SD, Hasko G, Lu Q, Xu DZ, Caruso JM, Sambol JT, Deitch EA (2002) Trauma/hemorrhagic shock mesenteric lymph upregulates adhesion molecule expression and IL6 production in human umbilical vein endothelial cells. Shock 17:491–495

Deitch EA (1998) Animal models of sepsis and shock: a review and lessons learned. Shock 9:1–11

Deitch EA, Livingston DH, Lavery RF, Monaghan SF, Bongu A, Machiedo GW (2007) Hormonally active women tolerate shock-trauma better than do men: a prospective study of over 4000 trauma patients. Ann Surg 246:447–453

Eachempati SR, Hydo L, Barie PS (1999) Gender-based differences in outcome in patients with sepsis. Arch Surg 134:1342–1347

Erol E, Kumar LS, Cline GW, Shulman GI, Kelly DP, Binas B (2004) Liver fatty acid binding protein is required for high rates of hepatic fatty acid oxidation but not for the action of PPARalpha in fasting mice. FASEB J 18:347–349

Esmon CT (2004) Why do animal models (sometimes) fail to mimic human sepsis? Crit Care Med 32:S219–S222

Finnerty CC, Herndon DN, Chinkes DL, Jeschke MG (2007) Serum cytokine differences in severely burned children with and without sepsis. Shock 27:4–9

Freise H, Bruckner UB, Spiegel HU (2001) Animal models of sepsis. J Invest Surg 14:195–212

Frink M, Pape HC, van Griensven M, Krettek C, Chaudry IH, Hildebrand F (2007a) Influence of sex and age on MODS and cytokines after multiple injuries. Shock 27:151–156

Frink M, Hsieh YC, Hsieh CH, Pape HC, Choudhry MA, Schwacha MG, Chaudry IH (2007b) Keratinocyte-derived chemokine plays a critical role in the induction of systemic inflammation and tissue damage after trauma-hemorrhage. Shock 28:576–581

Gabel SA, Walker VR, London RE, Steenbergen C, Korach KS, Murphy E (2005) Estrogen receptor beta mediates gender differences in ischemia/reperfusion injury. J Mol Cell Cardiol 38:289–297

Gannon CJ, Napolitano LM, Pasquale M, Tracy JK, McCarter RJ (2002) A statewide population-based study of gender differences in trauma: validation of a prior single-institution study. J Am Coll Surg 195:11–18

Gannon CJ, Pasquale M, Tracy JK, McCarter RJ, Napolitano LM (2004) Male gender is associated with increased risk for postinjury pneumonia. Shock 21:410–414

Gauglitz GG, Song J, Herndon DN, Finnerty CC, Boehning DF, Barral JM, Jeschke MG (2008) Characterization of the inflammatory response during acute and postacute phases after severe burn. Shock 30(5):503–507

George RL, McGwin G Jr, Metzger J, Chaudry IH, Rue LW III (2003a) The association between gender and mortality among trauma patients as modified by age. J Trauma 54:464–471

George RL, McGwin G Jr, Windham ST, Melton SM, Metzger J, Chaudry IH, Rue LW III (2003b) Age-related gender differential in outcome after blunt or penetrating trauma. Shock 19:28–32

Gruber CJ, Tschugguel W, Schneeberger C, Huber JC (2002) Production and actions of estrogens. N Engl J Med 346:340–352

Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman B, Montminy M (2001) CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. Nature 413:179–183

Hierholzer C, Harbrecht B, Menezes JM, Kane J, MacMicking J, Nathan CF, Peitzman AB, Billiar TR, Tweardy DJ (1998) Essential role of induced nitric oxide in the initiation of the inflammatory response after hemorrhagic shock. J Exp Med 187:917–928

Holcomb JB, Jenkins D, Rhee P, Johannigman J, Mahoney P, Mehta S, Cox ED, Gehrke MJ, Beilman GJ, Schreiber M, Flaherty SF, Grathwohl KW, Spinella PC, Perkins JG, Beekley AC, McMullin NR, Park MS, Gonzalez EA, Wade CE, Dubick MA, Schwab CW, Moore FA, Champion HR, Hoyt DB, Hess JR (2007) Damage control resuscitation: directly addressing the early coagulopathy of trauma. J Trauma 62:307–310

Hsieh YC, Yang S, Choudhry MA, Yu HP, Rue LW III, Bland KI, Chaudry IH (2005) PGC-1 upregulation via estrogen receptors: a common mechanism of salutary effects of estrogen and flutamide on heart function after trauma-hemorrhage. Am J Physiol Heart Circ Physiol 289: H2665–H2672

Hsieh YC, Yang S, Choudhry MA, Yu HP, Bland KI, Schwacha MG, Chaudry IH (2006a) Flutamide restores cardiac function after trauma-hemorrhage via an estrogen-dependent pathway through upregulation of PGC-1. Am J Physiol Heart Circ Physiol 290:H416–H423

Hsieh YC, Choudhry MA, Yu HP, Shimizu T, Yang S, Suzuki T, Chen J, Bland KI, Chaudry IH (2006b) Inhibition of cardiac PGC-1alpha expression abolishes ERbeta agonist-mediated cardioprotection following trauma-hemorrhage. FASEB J 20:1109–1117

Hsieh YC, Yu HP, Suzuki T, Choudhry MA, Schwacha MG, Bland KI, Chaudry IH (2006c) Upregulation of mitochondrial respiratory complex IV by estrogen receptor-beta is critical for inhibiting mitochondrial apoptotic signaling and restoring cardiac functions following trauma-hemorrhage. J Mol Cell Cardiol 41:511–521

Hubbard WJ, Choudhry MA, Schwacha MG, Kerby JD, Rue LW III, Bland KI, Chaudry IH (2005) Cecal ligation and puncture. Shock 24:52–57

Iafrati MD, Karas RH, Aronovitz M, Kim S, Sullivan TR Jr, Lubahn DB, O'Donnell TF Jr, Korach KS, Mendelsohn ME (1997) Estrogen inhibits the vascular injury response in estrogen receptor alpha-deficient mice. Nat Med 3:545–548

Iida M, Watanabe K, Tsurufuji M, Takaishi K, Iizuka Y, Tsurufuji S (1992) Level of neutrophil chemotactic factor CINC/gro, a member of the interleukin-8 family, associated with lipopolysaccharide-induced inflammation in rats. Infect Immun 60:1268–1272

Jarrar D, Wang P, Knoferl MW, Kuebler JF, Cioffi WG, Bland KI, Chaudry IH (2000a) Insight into the mechanism by which estradiol improves organ functions after trauma-hemorrhage. Surgery 128:246–252

Jarrar D, Wang P, Cioffi WG, Bland KI, Chaudry IH (2000b) The female reproductive cycle is an important variable in the response to trauma-hemorrhage. Am J Physiol Heart Circ Physiol 279:H1015–H1021

Kalaitzidis D, Gilmore TD (2005) Transcription factor cross-talk: the estrogen receptor and NF-kappaB. Trends Endocrinol Metab 16:46–52

Kamoun WS, Shin MC, Keller S, Karaa A, Huynh T, Clemens MG (2005) Induction of biphasic changes in perfusion heterogeneity of rat liver after sequential stress in vivo. Shock 24:324–331

Kanda N, Watanabe S (2004) 17beta-estradiol stimulates the growth of human keratinocytes by inducing cyclin D2 expression. J Invest Dermatol 123:319–328

Katznelson L, Riskind PN, Saxe VC, Klibanski A (1998) Prolactin pulsatile characteristics in postmenopausal women. J Clin Endocrinol Metab 83:761–764

Kawasaki T, Choudhry MA, Schwacha MG, Fujimi S, Lederer JA, Bland KI, Chaudry IH (2008) Trauma-hemorrhage inhibits splenic dendritic cell proinflammatory cytokine production via a mitogen-activated protein kinase process. Am J Physiol Cell Physiol 294:C754–C764

Kher A, Wang M, Tsai BM, Pitcher JM, Greenbaum ES, Nagy RD, Patel KM, Wairiuko GM, Markel TA, Meldrum DR (2005) Sex differences in the myocardial inflammatory response to acute injury. Shock 23:1–10

Kiang JG, Lu X, Tabaku LS, Bentley TB, Atkins JL, Tsokos GC (2005) Resuscitation with lactated Ringer solution limits the expression of molecular events associated with lung injury after hemorrhage. J Appl Physiol 98:550–556

Kirchhoff SR, Gupta S, Knowlton AA (2002) Cytosolic heat shock protein 60, apoptosis, and myocardial injury. Circulation 105:2899–2904

Knowlton AA, Sun L (2001) Heat-shock factor-1, steroid hormones, and regulation of heat-shock protein expression in the heart. Am J Physiol Heart Circ Physiol 280:H455–H464

Kobbe P, Vodovotz Y, Kaczorowski D, Mollen KP, Billiar TR, Pape HC (2008) Patterns of cytokine release and evolution of remote organ dysfunction after bilateral femur fracture. Shock 30(1):43–47

Kuebler JF, Jarrar D, Bland KI, Rue L III, Wang P, Chaudry IH (2003) Progesterone administration after trauma and hemorrhagic shock improves cardiovascular responses. Crit Care Med 31:1786–1793

Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138:863–870

Kumarapeli AR, Wang X (2004) Genetic modification of the heart: chaperones and the cytoskeleton. J Mol Cell Cardiol 37:1097–1109

Kushner PJ, Agard DA, Greene GL, Scanlan TS, Shiau AK, Uht RM, Webb P (2000) Estrogen receptor pathways to AP-1. J Steroid Biochem Mol Biol 74:311–317

Lin KM, Lin B, Lian IY, Mestril R, Scheffler IE, Dillmann WH (2001) Combined and individual mitochondrial HSP60 and HSP10 expression in cardiac myocytes protects mitochondrial function and prevents apoptotic cell deaths induced by simulated ischemia-reoxygenation. Circulation 103:1787–1792

Lindner V, Kim SK, Karas RH, Kuiper GG, Gustafsson JA, Mendelsohn ME (1998) Increased expression of estrogen receptor-beta mRNA in male blood vessels after vascular injury. Circ Res 83:224–229

Lobo R (2000) Menopause. In: Goldman L, Bennett J (eds) Cecil textbook of medicine. W.B. Sanders, Philadelphia, pp 1360–1366

MacConmara MP, Maung AA, Fujimi S, McKenna AM, Delisle A, Lapchak PH, Rogers S, Lederer JA, Mannick JA (2006) Increased CD4+ CD25+ T regulatory cell activity in trauma patients depresses protective Th1 immunity. Ann Surg 244:514–523

Malech HL, Gallin JI (1987) Current concepts: immunology. Neutrophils in human diseases. N Engl J Med 317:687–694

Meldrum DR, Shenkar R, Sheridan BC, Cain BS, Abraham E, Harken AH (1997) Hemorrhage activates myocardial NFkappaB and increases TNF-alpha in the heart. J Mol Cell Cardiol 29:2849–2854

Menezes JM, Hierholzer C, Watkins SC, Billiar TR, Peitzman AB, Harbrecht BG (2002) The modulation of hepatic injury and heat shock expression by inhibition of inducible nitric oxide synthase after hemorrhagic shock. Shock 17:13–18

Moore EE, Moore FA, Harken AH, Johnson JL, Ciesla D, Banerjee A (2005) The two-event construct of postinjury multiple organ failure. Shock 24(Supp 1):71–74

Mostafa G, Huynh T, Sing RF, Miles WS, Norton HJ, Thomason MH (2002) Gender-related outcomes in trauma. J Trauma 53:430–434

Murakami K, Enkhbaatar P, Yu YM, Traber LD, Cox RA, Hawkins HK, Tompkins RG, Herndon D, Traber DL (2007) L-arginine attenuates acute lung injury after smoke inhalation and burn injury in sheep. Shock 28:477–483

Nakano M, Mann DL, Knowlton AA (1997) Blocking the endogenous increase in HSP 72 increases susceptibility to hypoxia and reoxygenation in isolated adult feline cardiocytes. Circulation 95:1523–1531

Napolitano LM, Greco ME, Rodriguez A, Kufera JA, West RS, Scalea TM (2001) Gender differences in adverse outcomes after blunt trauma. J Trauma 50:274–280

Nishizawa J, Nakai A, Komeda M, Ban T, Nagata K (2002) Increased preload directly induces the activation of heat shock transcription factor 1 in the left ventricular overloaded heart. Cardiovasc Res 55:341–348

Offner PJ, Moore EE, Biffl WL (1999) Male gender is a risk factor for major infections after surgery. Arch Surg 134:935–938

Pandey P, Saleh A, Nakazawa A, Kumar S, Srinivasula SM, Kumar V, Weichselbaum R, Nalin C, Alnemri ES, Kufe D, Kharbanda S (2000) Negative regulation of cytochrome c-mediated oligomerization of Apaf-1 and activation of procaspase-9 by heat shock protein 90. EMBO J 19:4310–4322

Purcell EM, Dolan SM, Kriynovich S, Mannick JA, Lederer JA (2006) Burn injury induces an early activation response by lymph node CD4+ T cells. Shock 25:135–140

Reddy RC, Chen GH, Tekchandani PK, Standiford TJ (2001) Sepsis-induced immunosuppression: from bad to worse. Immunol Res 24:273–287

Remick DG, Ward PA (2005) Evaluation of endotoxin models for the study of sepsis. Shock 24 (Supp 1):7–11

Rossaint R, Cerny V, Coats TJ, Duranteau J, Fernandez-Mondejar E, Gordini G, Stahel PF, Hunt BJ, Neugebauer E, Spahn DR (2006) Key issues in advanced bleeding care in trauma. Shock 26:322–331

Schneider CP, Nickel EA, Samy TS, Schwacha MG, Cioffi WG, Bland KI, Chaudry IH (2000) The aromatase inhibitor, 4-hydroxyandrostenedione, restores immune responses following trauma-hemorrhage in males and decreases mortality from subsequent sepsis. Shock 14:347–353

Shanley TP, Schmal H, Warner RL, Schmid E, Friedl HP, Ward PA (1997) Requirement for C-X-C chemokines (macrophage inflammatory protein-2 and cytokine-induced neutrophil chemoattractant) in IgG immune complex-induced lung injury. J Immunol 158:3439–3448

Shi Y, Hutchins W, Ogawa H, Chang CC, Pritchard KA Jr, Zhang C, Khampang P, Lazar J, Jacob HJ, Rafiee P, Baker JE (2005) Increased resistance to myocardial ischemia in the Brown Norway vs. Dahl S rat: role of nitric oxide synthase and Hsp90. J Mol Cell Cardiol 38:625–635

Shimizu T, Yu HP, Suzuki T, Szalay L, Hsieh YC, Choudhry MA, Bland KI, Chaudry IH (2007) The role of estrogen receptor subtypes in ameliorating hepatic injury following trauma-hemorrhage. J Hepatol 46:1047–1054

Szalay L, Shimizu T, Schwacha MG, Choudhry MA, Rue LW III, Bland KI, Chaudry IH (2005) Mechanism of salutary effects of estradiol on organ function after trauma-hemorrhage: upregulation of heme oxygenase. Am J Physiol Heart Circ Physiol 289:H92–H98

Szalay L, Shimizu T, Suzuki T, Yu HP, Choudhry MA, Schwacha MG, Rue LW III, Bland KI, Chaudry IH (2006) Estradiol improves cardiac and hepatic function after trauma-hemorrhage: role of enhanced heat shock protein expression. Am J Physiol Regul Integr Comp Physiol 290: R812–R818

Tsujimoto H, Ono S, Efron PA, Scumpia PO, Moldawer LL, Mochizuki H (2008) Role of Toll-like receptors in the development of sepsis. Shock 29:315–321

Vincent JL (2000) Update on sepsis: pathophysiology and treatment. Acta Clin Belg 55:7–87

Voss MR, Stallone JN, Li M, Cornelussen RN, Knuefermann P, Knowlton AA (2003) Gender differences in the expression of heat shock proteins: the effect of estrogen. Am J Physiol Heart Circ Physiol 285:H687–H692

Wade CB, Dorsa DM (2003) Estrogen activation of cyclic adenosine 5'-monophosphate response element-mediated transcription requires the extracellularly regulated kinase/mitogen-activated protein kinase pathway. Endocrinology 144:832–838

Wang JL, Ke DS, Lin MT (2005) Heat shock pretreatment may protect against heatstroke-induced circulatory shock and cerebral ischemia by reducing oxidative stress and energy depletion. Shock 23:161–167

Wheeler DS, Lahni PM, Denenberg AG, Poynter SE, Wong HR, Cook JA, Zingarelli B (2008) Induction of endotoxin tolerance enhances bacterial clearance and survival in murine polymicrobial sepsis. Shock 30(3):267–273

Wichmann MW, Angele MK, Ayala A, Cioffi WG, Chaudry IH (1997) Flutamide: a novel agent for restoring the depressed cell-mediated immunity following soft-tissue trauma and hemorrhagic shock. Shock 8:242–248

Wichmann MW, Inthorn D, Andress HJ, Schildberg FW (2000) Incidence and mortality of severe sepsis in surgical intensive care patients: the influence of patient gender on disease process and outcome. Intensive Care Med 26:167–172

Wichterman KA, Baue AE, Chaudry IH (1980) Sepsis and septic shock – A review of laboratory models and a proposal. J Surg Res 29(2):189–201

Wohltmann CD, Franklin GA, Boaz PW, Luchette FA, Kearney PA, Richardson JD, Spain DA (2001) A multicenter evaluation of whether gender dimorphism affects survival after trauma. Am J Surg 181:297–301

Wu LL, Tang C, Liu MS (2001) Altered phosphorylation and calcium sensitivity of cardiac myofibrillar proteins during sepsis. Am J Physiol Regul Integr Comp Physiol 281:R408–R416

Yang S, Choudhry MA, Hsieh YC, Hu S, Rue LW III, Bland KI, Chaudry IH (2006) Estrus cycle: influence on cardiac function following trauma-hemorrhage. Am J Physiol Heart Circ Physiol 291:H2807–H2815

Yu HP, Yang S, Hsieh YC, Choudhry MA, Bland KI, Chaudry IH (2006a) Maintenance of lung myeloperoxidase activity in proestrus females after trauma-hemorrhage: upregulation of heme oxygenase-1. Am J Physiol Lung Cell Mol Physiol 291:L400–L406

Yu HP, Choudhry MA, Shimizu T, Hsieh YC, Schwacha MG, Yang S, Chaudry IH (2006b) Mechanism of the salutary effects of flutamide on intestinal myeloperoxidase activity following trauma-hemorrhage: up-regulation of estrogen receptor-{beta}-dependent HO-1. J Leukoc Biol 79:277–284

Yu HP, Shimizu T, Hsieh YC, Suzuki T, Choudhry MA, Schwacha MG, Chaudry IH (2006c) Tissue-specific expression of estrogen receptors and their role in the regulation of neutrophil infiltration in various organs following trauma-hemorrhage. J Leukoc Biol 79:963–970

Yu HP, Hsieh YC, Suzuki T, Shimizu T, Choudhry MA, Schwacha MG, Chaudry IH (2006d) Salutary effects of estrogen receptor-beta agonist on lung injury after trauma-hemorrhage. Am J Physiol Lung Cell Mol Physiol 290:L1004–L1009

Yu HP, Shimizu T, Choudhry MA, Hsieh YC, Suzuki T, Bland KI, Chaudry IH (2006e) Mechanism of cardioprotection following trauma-hemorrhagic shock by a selective estrogen receptor-beta agonist: up-regulation of cardiac heat shock factor-1 and heat shock proteins. J Mol Cell Cardiol 40:185–194

Yu M, Wang H, Ding A, Golenbock DT, Latz E, Czura CJ, Fenton MJ, Tracey KJ, Yang H (2006f) HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock 26:174–179

Zellweger R, Wichmann MW, Ayala A, DeMaso CM, Chaudry IH (1996) Prolactin: a novel and safe immunomodulating hormone for the treatment of immunodepression following severe hemorrhage. J Surg Res 63:53–58

Chapter 10
Sex Differences in Outcomes of Infections and Vaccinations in Under Five-Year-Old Children

Katie Louise Flanagan and Kristoffer Jarlov Jensen

Abstract It is evident that human male and female children differ in their outcomes following infectious challenge and vaccination. This chapter explores some of the evidence for this in children <5 years of age, which is the age group that suffers the greatest morbidity and mortality from infections, and the target age group for many vaccinations. The sex-differential effects commence both pre-implantation and in utero and continue throughout childhood. The mechanisms include genetic influences, epigenetic differences, the influences of sexually dimorphic hormones, sex differences in innate immunity, differences in thymic development, and sex differences in the effect of diet and breastfeeding. There may also be behavioral factors at play such as differential treatment of males and females. Studies in this age group are limited, and yet understanding the factors that determine key sex differences in immunity could lead to therapeutic strategies to improve childhood survival.

10.1 Sex Differences in Outcomes of Infections

It is newborns and infants under the age of 5 years that continue to suffer the bulk of infectious diseases in the world. In 2012, 6.6 million children under 5 years of age died, with the highest death rates occurring in sub-Saharan Africa where 1 in 10 children die before 5 years of age (WHO 2012). Up to a quarter of these deaths are caused by vaccine-preventable diseases including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Bordetella pertussis*, rotavirus, and measles, while

K.L. Flanagan (✉)
Department of Immunology, Monash University, Commercial Road, Prahran, Melbourne, VIC 3181, Australia
e-mail: katie.flanagan@dhhs.tas.gov.au

K.J. Jensen
Research Center for Vitamins and Vaccines, Statens Serum Institute, Copenhagen, Denmark
e-mail: KJJ@ssi.dk

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_10

Escherichia coli and other gram-negative organisms, group B streptococcus, respiratory syncytial virus, and herpes simplex virus also contribute a significant proportion. The majority of these deaths continue to occur in resource poor settings, while the developed world enjoys considerably better child health. The Millennium Development Goal 4 aims to decrease under 5-year childhood mortality by two thirds between 1990 and 2015 (UN 2013). Despite great progress in reaching this goal, efforts to decrease neonatal mortality (deaths in first 4 weeks of life) have proved more challenging, and the first 24 h of life continues to be the most vulnerable period accounting for almost half (44 %) of under-five deaths (UN 2013).

For decades, newborn males have anecdotally been considered more vulnerable to infections and death than females. More recently, modern epidemiology has been able to more systematically corroborate this observation in developing as well as developed countries (Sawyer 2012). However, the degree of excess male infant morbidity and mortality is subject to considerable geographical variation and may also depend on the nature of the local endemic diseases. Overall, the female advantage tends to become more evident as the mortality rate declines, in keeping with the fact that the decline in child mortality due to improved sanitary conditions and infectious diseases control in the industrialization era favored females more than males (Sawyer 2012; Drevenstedt et al. 2008). Notable exceptions are East/Southeast (E/SE) Asia and more developed countries (Fig. 10.1); possible explanations include preferential treatment of males compared to females or that at low child mortality rates in developed countries, the causes of death are less related to factors that vary between sexes, for example, more recent improvement in obstetric

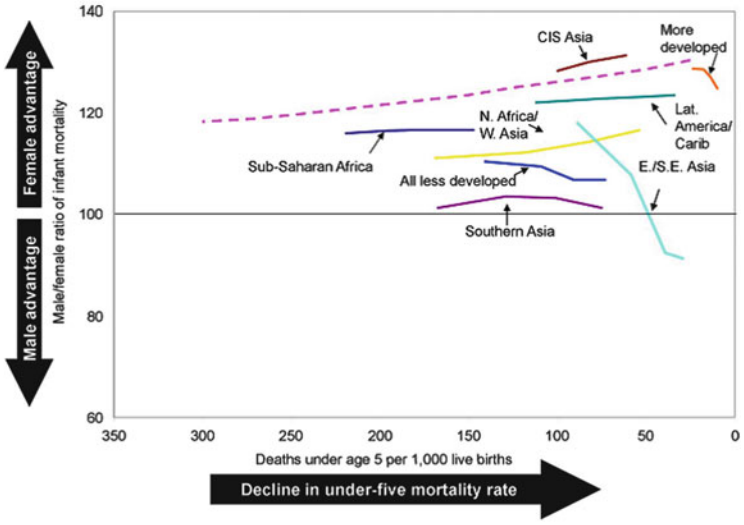

Fig. 10.1 Trends in the male-to-female ratio of infant mortality (ages 1–4 years) by level of under-five mortality in different regions of the world. The *dashed line in pink* is the historical sex ratio of infant mortality for selected developed countries [Reproduced from Sawyer (2012)]

practice and neonatal care has particularly favored males (Drevenstedt et al. 2008). The risk of contracting disease in childhood may often be age dependent in a sex-differential manner for a number of pathogens, with the prevailingly excess male case rate during early infancy shifting toward a higher female case rate later in childhood or adolescence (see Chap. 6).

Green postulated that male excess morbidity depends on the symptomatic to asymptomatic ratio for the specific disease, in the sense that severe infections such as measles with a high ratio (i.e., few asymptomatic infected) would manifest more equally in females and males, whereas diseases with a large proportion of asymptomatic infected individuals would materialize as a higher morbidity in the more vulnerable males (Green 1992). Birth defects are more frequently seen in males (Lary and Paulozzi 2001), and this may partly account for the increased susceptibility to some infections relative to females (Ulizzi and Zonta 2002).

10.1.1 Sex Differences in Susceptibility to Bacterial Infections and Sepsis in Childhood

Most studies show a higher susceptibility among males in the developed world to bacterial sepsis, including infants and preadolescent children (Watson et al. 2003), preterm infants (Neubauer et al. 2012), and newborns undergoing elective surgery for anomalies (Albers et al. 2002), while no sex difference was found in a cohort of extremely low-birth-weight infants (Stevenson et al. 2000). By contrast, a Nigerian study found a significantly higher incidence of bacterial sepsis among 1–5-year-old females compared to males, but only in the dry season (Omoregie et al. 2009). Similarly, among malaria-infected Kenyan children aged 1–3 years, bacteremia was more prevalent among females than males (Were et al. 2011). Such regional differences in the risk of septicemia for males and females may be due to differences in the endemic pathogenic bacteria species.

Several other murine and human studies show that prognosis during sepsis, shock, and trauma is better for females than males (see Chap. 9) (Angele et al. 2000; Marriott et al. 2006; Muller-Werdan et al. 2009). Watson and colleagues found significantly more sepsis cases and sepsis-related mortality among male than female infants in the USA (Hartman et al. 2013), with a persistently higher case rate but not mortality persisting to adolescence. Conversely, a smaller Dutch study of patients admitted to the pediatric intensive care unit for sepsis and purpura failed to find a difference in the case fatality rate according to sex, although males did suffer more severe disease (Maat et al. 2007). Low-birth-weight (LBW) males also had higher hospital mortality than LBW females in this study (Watson et al. 2003). In a prospective Austrian study of all infants born at <32 weeks gestational age, females suffered less from early onset sepsis (<72 h from birth) and respiratory infections than males, with a lower rate of hospitalization of females in the first year but no sex difference in the second year (Neubauer et al. 2012).

In a study of very low-birth-weight infants (i.e., 500–1,500 g) conducted in the USA, males were more premature, had lower Apgar scores (medical evaluation routinely performed at birth for appearance, pulse, grimace, activity, and respiration), and had 50 % higher mortality than females. Males suffered more from urinary tract infections than females and were more in need of intensive care and medication, while rates of septicemia and meningitis were similar in males and females (Stevenson et al. 2000). Similarly, Ghuman et al. found comparable mortality and severity of sepsis in prepubertal male and female children (2–7 years old) admitted to hospitals in the USA with sepsis, while postpubertal males had higher mortality due to more severe illness (Ghuman et al. 2013).

Males seem more susceptible to diarrheal diseases than females. In four geographically different highly industrialized nations (Canada, Scotland, New Zealand, and Norway), campylobacter infection incidence was significantly higher in male than female infants and 1–5-year-old children, and the excess male case ratio reverted after adolescence (Strachan et al. 2008). A mouse study showed that after inoculation with *Campylobacter jejuni*, male mice were more heavily colonized, shed a larger number of bacteria in feces, and presented with a more systemic infection that spread to tissues as compared with females (Strachan et al. 2008). A community cohort study from West Africa further found that male sex was a significant risk factor for diarrhea (all causes) among 0–3-year-old children (Molbak et al. 1997). Sudanese <5-year-old males also had a slightly (3 %) but significantly higher risk of having diarrhea than age-matched females (Siziya et al. 2013).

The incidence of invasive pneumococcal infection was higher in Danish males than females 2–6 years of age, and the incidence of otitis media (which can have both bacterial and viral etiology) was higher among males than females in Finnish under 2-year-old children (Seppala et al. 2011). A large registry-based study from Japan found a male preponderance for group A streptococcal pharyngitis and enterohemorrhagic *E. coli* infection among young children, with a female dominance for *Mycoplasma pneumoniae* and *Bordetella pertussis* infections (Eshima et al. 2012). Pulmonary tuberculosis (TB) is more prevalent in adult males of all ages, whereas in children and early adolescents, there is no sex preponderance (see Chap. 8) (Neyrolles and Quintana-Murci 2009).

10.1.2 Sex Differences in Viral Infections in Childhood

The aforementioned registry-based study from Japan showed that for most of the investigated viral diseases, there was an excess male morbidity ratio in infancy which reversed to an excess female ratio during adolescence, although not consistently for all viral infections (Eshima et al. 2012). During an outbreak of measles in Korea, more male than female children were hospitalized (Lee et al. 2007), and in an outbreak in Ireland, slightly but nonsignificantly more male children were hospitalized with measles (McBrien et al. 2003). However, a survey in India

found that nearly 50 % more females than males aged 1–59 months succumbed to measles (Morris et al. 2013). In a Zambian study, the total lymphocyte count in children with measles was significantly higher in males, and the CD4:CD8 ratio was higher in females during recovery, alongside a more pronounced and prolonged lymphopenia in females than males (Ryon et al. 2002). Surface expression of Fas on lymphocytes or soluble Fas was not different between males and females. Among measles, mumps, and rubella (MMR)-vaccinated older children/young adults (11–22 years), females responded with significantly stronger TNF-α, IL-6, and IFN-α responses to measles antigen than males (Umlauf et al. 2012).

Danish female infants (relative risk (RR): 0.56 (0.45–0.69)) and female children aged 1–14 years (RR: 0.45 (0.40–0.51)) had a lower risk of being hospitalized for viral meningitis than males (Hviid and Melbye 2007). Another Danish registry study of under 5-year-old children found that the relative risk of being hospitalized for four categories of respiratory tract infection (i.e., influenza, middle ear infection, pneumonia, acute upper respiratory tract infection) was higher for males than females for all four diagnoses (Jensen-Fangel et al. 2004). In a Canadian hospital cohort, male sex was identified as a risk factor for being hospitalized with a diagnosis of seasonal influenza (OR: 1.9 (1.0–3.7)) for children under 5 years of age (Quach et al. 2003). In a very low-birth-weight cohort, sex was not a determinant for the risk of human rhinovirus-associated respiratory infection (Miller et al. 2012). In Japan, more male than female neonates were hospitalized with RSV bronchiolitis, and more males than females under 6 months of age were febrile, while the opposite was found for infants more than 6 months of age (Nagayama et al. 2006).

According to a registry-based study among 0–14-year-olds from the UK, female children seem to be more susceptible to recurrent herpes virus infections, including shingles which is caused by recurrence of latent varicella zoster and herpes simplex virus infection which remains latent and becomes reactivated from time to time (Fleming et al. 2004). No sex difference was observed for chickenpox, the primary herpes zoster infection.

10.1.3 Sex Differences in Parasitic Infections in Childhood

The prevalence and intensity of most parasitic infections is higher among male than female children and adults (Klein 2004). Most human childhood studies have been of school-age children, with few studies describing sex differences in parasite levels in infants <5 years of age. Those infections shown to be higher in school-age male children include the human protozoal infection *Plasmodium falciparum*, the trematode *Schistosoma mansoni,* and the nematodes *Necator americanus* (hookworm), *Toxocara* spp., and *Wuchereria bancrofti*. Human males <15 years of age are more susceptible to toxoplasma lymphadenopathy than females, whereas among sexually mature adults >15 years, the females were the ones more susceptible, suggesting a switching of the sex effect in puberty (Beverley et al. 1976). A study of imported

malaria cases in Germany suggested that both preadolescent and adolescent males experience higher malaria parasitemia than females (Weise 1979), and male Ghanaian schoolchildren similarly had higher parasitemias than females (Landgraf et al. 1994). By contrast, a study from an area with a low prevalence of malaria found no sex bias for preadolescent children, while the incidence rate increased relatively more among males than females after adolescence (Pathak et al. 2012). Prepubertal males are more likely to develop visceral leishmaniasis than females (Shiddo et al. 1995). Among under 2 years old West African children, infection with *Cryptosporidium parvum* imposed a higher risk of diarrhea in males than females (Valentiner-Branth et al. 2003), while the prevalence of *Giardia lamblia* and *Entamoeba histolytica* was higher in under 5-year-old females from Guinea-Bissau presenting with diarrhea compared to males in a study of almost 5,000 stool samples (Perch et al. 2001). A higher relative fecal load of *Schistosoma mansoni* eggs was found among Ethiopian male schoolchildren compared to females (Degu et al. 2002) and Senegalese of all ages from 8 years to adulthood (Marguerite et al. 1999). Thus, the human data of childhood susceptibility to parasitic infection supports a male bias for most infections, as seen in adults. See also Chap. 13 for more information on sex differences in parasitic diseases.

10.2 Sex Differences in Immune Responses to Vaccinations in Childhood

Worldwide vaccination of infants has been a resounding success in the effort to reduce mortality and morbidity from infections in infants and children. However, recipients are not equal in how they respond to vaccines, and although often disregarded in vaccination policy and vaccine trials, sex may partly determine vaccine outcome.

10.2.1 Sex differences in Antibody Responses to Vaccines

In adults, sex differences in responses to vaccination have been reported for most commercially available vaccines (Klein and Poland 2013), including live vaccines such as measles vaccine, yellow fever vaccine, Venezuelan equine encephalitis vaccine, and rubella vaccine and inactivated vaccines including those against hepatitis A and B, influenza, tetanus, and rabies (Cook 2008). In children, male/female differences in antibody responses seem to vary according to the vaccine, most studies being of responses to routine childhood vaccines, particularly the measles vaccine (MV).

In Guinea-Bissau, pre-vaccination anti-measles antibody levels at 4.5 months of age were lower in females than males. However, at 9 months of age, non-vaccinated

females were more likely to have protective anti-MV antibody levels than males. The study suggested that females were more likely to have had a subclinical measles infection during the first 9 months of life (Martins et al. 2009). Similarly, a study in Pakistani infants found higher seropositivity rates and serum titers in females prior to MV at 9 months of age (Hussain et al. 2013).

After standard MV administered at 9 months of age, antibody levels were higher in females than males at 18 months of age in Guinea-Bissau, but only for the Edmonston-Zagreb (EZ) strain vaccine, while there was no sex difference for the Schwarz strain MV (Martins et al. 2013). In a study designed to explore sex differences in MV immunization responses in infants, among those with undetectable pre-immunization anti-MV titers, females responded with antibody of lower specific antibody-dependent cell-mediated cytotoxicity (ADCC) activity than males after EZ MV, but not after Schwarz MV (Atabani et al. 2000).

Indonesian females were found to have lower antibody responses than males following MV at 9 months of age (Semba et al. 1995), and in Pakistan, 5-year-old females also had lower measles seropositivity rates and serum titers than males (Hussain et al. 2013). By contrast, Tanzanian <5-year-old females had higher mean anti-measles antibody titers than males after standard MV (Lyamuya et al. 1999). In a multiple logistic regression model, sex was not a factor influencing the risk of having non-detectable measles antibody titers (Lyamuya et al. 1999), and there were no sex differences in MV antibody levels to an MV booster at 36 months of age in the Gambia (Njie-Jobe et al. 2012). Benn et al. found that in Guinea-Bissau, simultaneous vitamin A supplementation increased antibody responses to MV in males, but not in females. However, among infants not receiving vitamin A, antibody titers after MV were higher in females (Benn et al. 1997).

Antibody levels after diphtheria vaccine were higher in Gambian female infants than in male infants (Moore et al. 2006), while males had higher antibody responses than females following a diphtheria booster in adolescence (Mark et al. 1999). In Chinese children up to 12 years old, females responded with higher antibody levels to hepatitis B vaccination than males (Fang et al. 1994). The lower titers in males may be associated with the finding of a higher preponderance in hepatitis B cases among hepatitis B-vaccinated Taiwanese infants (Chen et al. 2004). There were no sex differences in antibody titer response to pertussis vaccination (whole cell or acellular) in American infants (Christy et al. 1995) or in responses to polyvalent meningococcal group A or group C vaccines in 4–8-year-old Nigerian children (Mohammed and Damisah 1982). For hepatitis A-vaccinated children aged 3–6 years, antibody responses were higher in females than males but significantly so only after the second dose (McMahon et al. 1995) suggesting that the number of doses might play a role. The persistence of antibodies after primary vaccination may be different in males and females, which in turn may influence the booster response (Cook 2008), but further studies are needed to investigate this.

The sex effect for responses to a particular vaccine may be different in children and adults. For example, Indonesian males had higher antibody responses following measles vaccination at 9 months of age (Semba et al. 1995), while females mounted higher antibody responses when vaccinated as adults (Green et al. 1994). In

children aged 6–9 years, females had higher antibody levels than males to pneumococcal vaccine and intramuscular (i.m.) rabies vaccination (Moore et al. 2003), while adult males had a greater antibody response to i.m. rabies vaccine than adult females (Siddiqui et al. 2001).

The above studies suggest that multiple factors may modulate antibody responses to childhood vaccines in a sex-differential manner, including age, vaccine type, micronutrient supplementation, and factors related to geographic setting.

10.2.2 Sex Differences in Vaccine-Induced Cellular Immunity

Few studies have investigated sex differences in cellular responses after vaccination in children. With respect to BCG vaccination, the tuberculin skin test (TST) response (Roth et al. 2005) and scar formation at the site of injection (Burl et al. 2010), both which serve as crude immunological markers of vaccine efficacy, have been shown to be larger in males, while other studies showed that the likelihood of having a positive TST was not affected by sex (Okan et al. 2006). A study of cytokine responses (IFN-γ, IL-5, and IL-13) following BCG vaccination in 236 Gambian infants found no evidence of sex differences in responses to several mycobacterial antigens 2 months after vaccination at birth (Finan et al. 2008). The authors did point out the striking interindividual variation in cytokine responses in this study, and this would likely hamper the likelihood of detecting significant sex differences. Similarly, there were no significant sex differences on type 1 and 2 cytokine responses to purified protein derivative from *M. tuberculosis* (PPD) in infants receiving oral polio vaccine together with BCG at birth (Sartono et al. 2010).

There were no sex differences in measles-specific IFN-γ or IL-4 responses after measles, mumps, and rubella (MMR) vaccination (Dhiman et al. 2005), and the immunosuppression characteristically seen after measles vaccine was similar in male and female infants in another study (Hussey et al. 1996). Cellular responses as measured by IFN-γ ELISpot assay following a booster measles vaccination at 36 months of age did not vary with sex (Njie-Jobe et al. 2012), and differential expression of cellular activation markers after measles vaccine was also unaffected by sex (Schnorr et al. 2001). A randomized trial of measles vaccine in 4.5-month-old infants from Guinea-Bissau found that vaccination increased plasma levels of IL-1 receptor agonist, IL-8, and MCP-1 in females but not in males, whereas there was no sex difference in the MV effect on in vitro cytokine responses (Jensen et al. 2014).

Overall, these studies do not support major sex differences in cell-mediated immune responses to vaccines in infants, although given the paucity of data, this needs to be confirmed by further studies. This contrasts to adults where cytokine responses following influenza vaccination were higher in females than males

(Furman et al. 2014), and vaccinia-specific IFN-γ ELISpot and IL-1β responses were higher in smallpox-vaccinated males, while vaccinated females had higher secretion of vaccinia-specific IL-2 and IL-10 (Haralambieva et al. 2013).

10.2.3 Sex Differences in Reactogenicity/Adverse Events to Vaccination

Adverse reactions to vaccines include allergic reactions that can be local or systemic and vaccine-induced autoimmune reactions. There have been a number of reports showing that females suffer greater reactogenicity and adverse events to vaccination than males. However, the sex bias may be age dependent. Females experienced more adverse events to oral poliovirus vaccination during mass immunization of high-school and university students in the Democratic Republic of the Congo at a ratio of 2 females for every male (Nzolo et al. 2013). A study in pediatric hospital patients found that females had a higher risk than males of experiencing an adverse drug reaction (ADR) to an unspecified group of drugs including, but not exclusively, vaccines (Martinez-Mir et al. 1999). Another study found a preponderance of male ADR reports among pediatric patients, in contrast to an excess female ADR rate among the adult population, with vaccines and immunoglobulins (37 %) being the largest drug group associated with the ADR reports (Ribeiro-Vaz et al. 2013). A similar male predominance in adverse reaction ratio in infancy declining over age and switching to a female predominance from adolescence has also been described for non-vaccine-related adverse events (Star et al. 2011). In a 6-month follow-up telephone survey of 946 children aged 0–14 years receiving vaccines, more adverse events were reported for males, although this was not significant, and adverse events by vaccine type was not analyzed by sex (Carrasco-Garrido et al. 2004). For yellow fever vaccine, a higher incidence of postvaccination encephalitis has been described in infant males compared to females (Cook 2008). Similarly, a higher serious adverse event rate in males compared with females after yellow fever vaccination was also reported in adults in one study (Lindsey et al. 2008), but not for smallpox vaccination (Reif et al. 2008). Israeli female toddlers experienced significantly higher risk of fever and rash following MMR vaccination (Shohat et al. 2000), whereas no sex differences in reactogenicity to MMR were reported in a Finnish study (Virtanen et al. 2000). There was no sex difference in rash after high-titer measles vaccine in Senegal, but there was an association between rash and mortality after the vaccine, perhaps stronger in females, but not significantly sex dependent (Seng et al. 1999).

The above studies suggest that infant males might fare worse than female infants after vaccination, but this might then switch with an increase in adverse events occurring in adolescent and adult females. This would suggest a hormonal influence in adverse events to vaccines although this has not been investigated to date.

10.2.4 Sex Differences in the Nontargeted Heterologous Effects of Vaccines

It is now evident that in addition to inducing vaccine-specific immunological memory, vaccines can also have more generalized effects on the immune system which alter susceptibility to non-vaccine-related infections leading to altered morbidity and mortality. The first descriptions of these effects, originally called "nonspecific effects" of vaccines, came from observational studies mainly in West Africa (Shann 2010). More recently the term "heterologous effects" has been adopted to describe this phenomenon (Flanagan et al. 2013). Randomized controlled trials are now being conducted which confirm these findings, and the immunological mechanisms are beginning to emerge. Interestingly females are generally more susceptible to the heterologous immunological effects of vaccines than are males (Flanagan et al. 2011), and some of the potential mechanisms for this sex difference will be explored in this section.

10.2.4.1 Sex Differences in the Heterologous Effects of Bacillus Calmette–Guérin Vaccine

To date, the tuberculosis (TB) vaccine bacillus Calmette–Guérin (BCG) remains the most commonly used vaccine in the world. It is recommended that BCG be given to neonates or at the first possible opportunity in the first year of life in TB endemic areas of the developing world. BCG is protective against disseminated TB in childhood but has variable protective efficacy in adults.

Studies in the early 1960s in the UK and USA showed that BCG-vaccinated children had lower mortality from non-TB causes than those that were BCG naïve, well beyond any effect on TB-related mortality (Roth et al. 2006). This led to the design of several randomized controlled trials aimed at investigating the effect of neonatal BCG vaccination on infection rates and non-TB-related mortality. BCG vaccination at birth of low-birth-weight (LBW) neonates has been shown in a randomized trial to reduce all-cause mortality in the vulnerable neonatal period by 45 % in a high-disease-burden setting in West Africa (Aaby et al. 2011). This study showed a sex-differential effect of neonatal BCG vaccination whereby males experienced a very rapid improvement in survival within 3 days of BCG vaccination, whereas the protective effects were only apparent in females after 1 week (Biering-Sorensen et al. 2012). Furthermore, BCG was found to reduce acute lower respiratory tract infections in female, but not male infants (Stensballe et al. 2005). Therefore, BCG vaccination seems to modify the immune system in a beneficial way, providing protection against nontuberculous infections and thereby reducing all-cause mortality, sometimes in sex-specific ways.

10.2.4.2 Sex Differences in the Heterologous Effects of Measles Vaccine

Measles vaccine (MV), which has been in wide use since the 1960s (Baker 2011), has also been shown to have heterologous survival benefits against nontargeted infections. It reduces the overall morbidity and mortality to an extent that exceeds the morbidity and mortality possibly caused by measles infection (Aaby et al. 1995, 2010b). Several observational studies from different countries, including Guinea-Bissau, Malawi, and India, have found that females benefit more than males from this beneficial heterologous effect of MV (Aaby et al. 1995, 2006c, 2010a; Hirve et al. 2012). The administration of MV at 4.5 months of age was found to reduce mortality among females, but not males. Furthermore, males that had received neonatal vitamin A supplementation had decreased survival after MV (Aaby et al. 2010b). The MV efficacy against hospitalization was better for females than males for those who had received MV as the last vaccine (Aaby et al. 2010a). A study in rural Malawi showed that either BCG or MV as the last vaccine was associated with a lower female to male mortality rate ratio (Aaby et al. 2006c).

10.2.4.3 Excess Female Mortality Following High Titer Measles Vaccine

While the standard measles vaccine has beneficial effects on infant survival, the high-titer measles vaccine (HTMV) introduced in 1989 was found in randomized trials to be associated with increased mortality among vaccinated females (Aaby et al. 1994; Holt et al. 1993), leading to the withdrawal of HTMV by WHO. The rationale behind using this more immunogenic vaccine in high measles endemic settings was to allow earlier vaccination in the presence of higher levels of maternally acquired antibodies, believed to impair vaccine efficacy. This was the first widely accepted evidence that vaccines could affect all-cause mortality in a sex-dependent manner.

10.2.4.4 Heterologous Effects of Diphtheria, Tetanus, and Whole-Cell Pertussis Vaccine

While BCG and measles vaccine (MV) seem to improve morbidity and mortality rates, particularly among females, the opposite is true for the combined diphtheria, tetanus, and whole-cell pertussis (DTwP) vaccine. DTwP vaccination is associated with higher female morbidity and mortality, but this is not the case for males (Agergaard et al. 2011; Aaby et al. 2007c, 2012a, b). Indeed, a re-analysis of the data from the high-titer measles vaccine (HTMV) studies showed that the increased mortality among female infants was more likely due to DTwP being given after HTMV (Aaby et al. 2003, 2006a). A study in the Philippines did not find a negative effect of DTwP on survival, but did find an indication of a sex-differential effect,

with the largest positive effect on survival being in the males (Chan et al. 2007). Recently, hepatitis B vaccine (HBV) and *Haemophilus influenzae* type b (Hib) were added to the DTwP vaccine and are now given in a multidose schedule as a pentavalent vaccine formulation to infants in many parts of the developing world. The pentavalent vaccine has not been tested for deleterious heterologous effects.

It has become clear that the order in which vaccines are given is crucial in the context of heterologous effects, with the last vaccine given determining the predominant effect. For example, the measles vaccine (MV) efficacy against hospitalization of 6–59-month-old children was lost among females if DTwP was given after MV (Aaby et al. 2010a). An observational study in India showed that the female–male mortality ratio up to 5 years of age differed according to the age group in which different vaccines were given: with a reduced ratio when BCG or MV were the latest vaccine and increased ratio when DTwP was the most recent vaccine (Hirve et al. 2012). Similarly, a study from the Gambia showed that females had a lower mortality rate than males throughout the age span from birth to 5 years of age, except for 6–8 months of age coinciding with the period when DTwP was the last vaccine given. In contrast, BCG and MV were usually the last vaccines received before and after, respectively, the intermittent DTwP period (Aaby et al. 2006b). A study of the hospital case fatality rates for male and female children 6–17 months of age showed that DTwP after measles vaccination was associated with an increased female–male fatality rate ratio, whereas DTwP followed by MV showed the opposite trend, although it was not significant (Aaby et al. 2007b). The relative risk for males and females of having rotavirus-associated diarrhea also depends on whether DTwP or BCG is the last vaccine, with DTwP vaccination being associated with an increased risk of rotavirus-associated diarrhea in females only (Rodrigues et al. 2006). The same trend was found for diarrhea caused by other pathogens.

WHO commissioned a number of investigations of the effect of DTP on all-cause mortality. None of the resulting reports found negative effects of DTP. However, a controversy ensued over the methodology applied in these studies, as the handling of missing vaccine information in the statistical models may have inflicted a bias on the results (Fine and Smith 2007; Aaby et al. 2007a; Jensen et al. 2007). The controversy remains unresolved.

10.2.4.5 Other Vaccines and Sex-Differential Heterologous Effects

A possible sex-differential effect of oral polio vaccine (OPV) was suggested in an observational study (Benn et al. 2008b), although a later randomized trial of OPV at birth did not support this (Lund et al. 2012). The female–male mortality ratio was higher in HBV-vaccinated than HBV-non-vaccinated infants (7.5–12 months of age), suggesting a deleterious effect among females, a beneficial effect for males, or both (Garly et al. 2004).

10.3 Mechanisms of Sex Differences in Infections and Vaccine Responses in Children

The biological explanations for the epidemiological findings remain to be fully elucidated. In general, studies of sex differences in immunity are lacking or sporadic, largely due to the fact that animal studies are generally conducted in either males or females, but not in both (Flanagan 2014). Furthermore, many adult human studies are carried out in men only since women have the risk of becoming pregnant and the monthly menstrual cycle might alter immune responses.

There are even less studies on the mechanisms of sex differences in immunity in infants and children. This has been largely overlooked probably because it has always been assumed that prepubertal children are not influenced by sex hormones. Furthermore, infant blood volumes available are small and immunological studies are more difficult to carry out. Herein we will review what is known regarding potential mechanisms for sex differences in children <5 years old.

10.3.1 Sexually Dimorphic Hormones in Early Life and Childhood

The immunological functions of individual sex hormones are described in detail in Chap. 1. While the hormonal status of newborn infants is relatively immature, there are key sex differences in early life that might contribute to sexual dimorphism in immunity, although hormonal differences are unlikely to fully account for sex differences in immunity in prepubertal children. Distinct sex differences in gonadotrophin levels are observed in the first 4 years of life (Winter et al. 1975). Both males and females have a postnatal rise in leutinizing hormone (LH) peaking at 1 month and follicle-stimulating hormone (FSH) peaking at 2–3 months. The female FSH peak is higher and more sustained than in males, whereas the LH peak is higher in males and declines to usual childhood levels by 4 months of age (Winter et al. 1975). Males have an early testosterone surge which peaks at 2–3 months of age (Andersson et al. 1998; Forest et al. 1973), and during this time, females generally have higher estrogen levels than males (Ikegami et al. 2001; Ji et al. 2008) (Fig. 10.2). Almost all cells including cells of the immune system have intracellular receptors for sex steroid hormones (Choudhry et al. 2007). Cord blood mononuclear cells (CBMC) express higher levels of the estrogen and progesterone receptors than adult peripheral blood cells, which is thought to make these cells more sensitive to the estrogen- and progesterone-mediated inhibition of innate inflammatory responses to bacterial stimuli (Giannoni et al. 2011).

Animal studies suggest that hormonal imprinting may occur early in life since prepubertal gonadectomy led to a loss of female resistance to *Brugia malayi* infection in mice, whereas adult gonadectomy has no such effect (Rajan et al. 1994). The early hormonal differences in human male and female infants

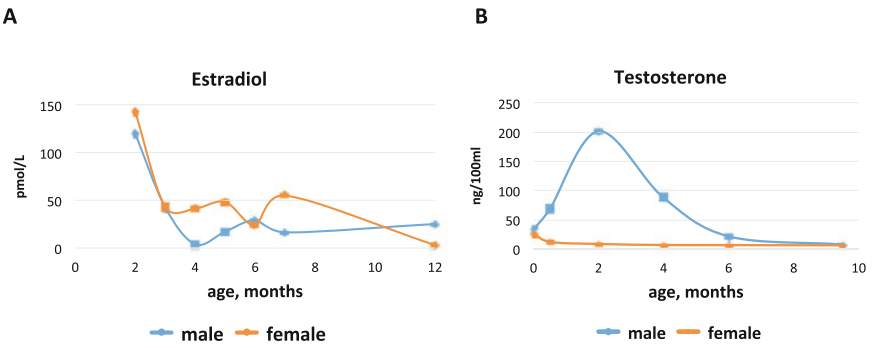

Fig. 10.2 Sex hormone levels in female and male infants. Figure showing the variations in estradiol (**a**) (from Ji et al. 2008) and testosterone (**b**) (from Forest et al. 1973) in human infants in the first 12 months of life. Estradiol levels are generally higher in females, while infant males have a testosterone surge at 2–4 months of age

may therefore have a long-lasting impact on sex-differential disease susceptibility. Casimir et al. examined for sex differences in inflammatory responses in normal prepubertal children as young as 5 months old compared to Turner's syndrome (TS) children (genotype XO), primarily to assess for the relation to X chromosome monosomy (Casimir et al. 2010a). Males had higher inflammatory responses than females to lipopolysaccharide (LPS) and pokeweed mitogen (PWM), and TS patients followed the male pattern providing evidence for the role of X inactivation in females and absence in males in the lifelong sexual dimorphism in immunity.

10.3.2 Sex Differences in Immune Activation

Among infants hospitalized with respiratory syncytial virus (RSV) bronchiolitis, more males than females <6 months of age were febrile, which was opposite for infants >6 months old. Blood eosinophilia was more common in males than females <4 months old, whereas total white blood cell counts and C reactive protein (CRP) were higher in females (Nagayama et al. 2006). In a cohort of 482 children <10 years of age hospitalized for various conditions, CRP, erythrocyte sedimentation rate (ESR), and neutrophil counts were all higher in females than males (Casimir et al. 2010b). Furman and colleagues found higher levels of serum leptin, IL-1 receptor antagonist, CRP, granulocyte macrophage colony-stimulating factor (GM-CSF), and IL-5 in adult females compared to males, while CRP, IL-6, and GM-CSF correlated with baseline levels of phosphorylated STAT3 proteins in adult female peripheral monocytes, supporting higher baseline inflammation among adult females (Furman et al. 2014). By contrast, another study in healthy human adults found that the endotoxin-induced CRP response was higher in males than females (Ferguson et al. 2013). The above evidence tends to suggest that

females are more inflammatory during childhood and adulthood, but a systematic analysis of this has not been carried out. African origin individuals had higher baseline inflammation than those of European origin (Ferguson et al. 2013), suggesting that children resident in high disease-endemic settings may start at higher baseline immune activation levels than those in the developed world; thus, the study area may influence the results in immune activation studies.

10.3.3 Sex Differences in Innate Immunity in Childhood

The innate immune system is required for the rapid defense against invading microorganisms and is mediated by factors including complement activation, cell lysis, and phagocytosis involving granulocytes, monocyte/macrophages, dendritic cells, and NK cells. The newborn infant is highly reliant on innate defenses for protection against infectious challenge since they have poorly developed adaptive immunity with minimal immunological memory. However, the innate immune system is not fully developed at birth either, and full capacity is not achieved until teenage years for some functions. NK cells have less potent cytotoxic activity (Guilmot et al. 2011); neonatal neutrophils are functionally suboptimal (Carr 2000); and complement components are up to 70 % lower than in adults at birth, but rapidly reach adult levels (Levy 2007). One of the few studies to describe sex differences in innate cells in human infants reported higher monocyte counts in males compared to females at 2 and 13 months of age and higher basophil counts in males at 13 months of age (Bellamy et al. 2000); another study reported higher NK cell frequencies in male children, although the differences were relatively small (Lee et al. 1996).

Innate cells express pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), which detect highly conserved pattern-associated molecular patterns (PAMPS) expressed by the invading pathogen or vaccine. Expression of PRRs on immune cells is fully developed at birth, and yet reactivity to these sensors is generally low at birth and progressively develops in the first few years of life (Levy et al. 2004; Burl et al. 2011; Kollmann et al. 2009; Belderbos et al. 2009; Nguyen et al. 2010). Production of the Th17-polarizing cytokines IL-6 and IL-23 dominates the innate response at birth (Angelone et al. 2006) and declines in the first 2 years of life, while TNF-α and IL-1β production rises (Burl et al. 2011; Kollmann et al. 2009; Belderbos et al. 2009; Nguyen et al. 2010). Human neonatal antigen-presenting cells (APC) and monocytes are at lower than adult levels at birth and are functionally suboptimal (De Wit et al. 2004; Aksoy et al. 2007), but mature to adult functional levels by 9 months of age (Nguyen et al. 2010), while the diminished IL-12 expression (Goriely et al. 2004) reached adult levels by 9 months of age in one study [188], but remained below adult levels at 2 years of age in another study (Corbett et al. 2010). Several innate signalling pathways show low activity at birth (Danis et al. 2008; Aksoy et al. 2007; Yan et al. 2004). Few studies have investigated for sex differences in

innate immunity in infants. In one study, infant males had more robust proinflammatory responses to low concentrations of LPS compared to females, but the effect narrowed at higher LPS concentrations (Casimir et al. 2010a). In the same study, infant males produced more IL-1 and IL-6 to the monocyte stimulus pokeweed mitogen than females (Casimir et al. 2010a). Overall, the studies support robust innate immunity from shortly after birth that is different to the pattern observed in adults, but adapted to the needs of early life. Innate cytokine responses to PRR stimulation develop to adult level by 1 year of age for most, but not all, cytokines, and infant males may have more robust innate immunity compared to females, but further studies are required to confirm this.

10.3.4 Sex Differences in Thymic Development and Adaptive Immunity in Childhood

Most of the observations regarding sex differences in adaptive immunity have been made in animal studies or human adults, and may not apply to neonates and infants who have quite different adaptive immune systems (Kollmann et al. 2012). One study in Guinea-Bissau found that female children aged 3–13 years had higher rates of skin test anergy than males to proteus, trichophyton, candida, tetanus, diphtheria, streptococcus, and tuberculin, suggesting poorer cell-mediated immunity in female children (Shaheen et al. 1996).

Sex hormones and their influence on adaptive immunity, for example, the Th1/Th2 profile, have been described previously (Chap. 1). The thymus is critical for maintaining the peripheral T-cell pool and thus plays a pivotal role in adaptive immunity. The survival of infants in West Africa (Aaby et al. 2002; Garly et al. 2008) and Bangladesh (Moore et al. 2014) correlates with thymus size, with a small thymus being a predictor for death. In the latter study, this effect was apparent at 8 weeks of age but not at birth suggesting postnatal influences; indeed longer exclusive breastfeeding, season, and male sex have all been associated with larger thymic size (228). Male rats have greater thymic weight and cellularity than female rats and different distributions of $CD4^{-}CD8^{-}$, $CD4^{+}CD8^{-}$, and $TCR\alpha\beta^{+}$ T cells which are altered by gonadectomy suggesting sex steroid influences on thymocyte composition (Leposavic et al. 1996).

Hypothalamo–pituitary–gonadal (HPG) axis signals (sex steroids and gonadotrophins) play a role in programming the kinetics of thymic maturation/involution partly accounting for early sexual immunologic dimorphism. Neonatal androgenization experiments in rodents demonstrate that the neonatal steroid milieu is important in programming sexual differences in thymopoietic efficiency, leading to sex differences in the peripheral T-cell compartment (Leposavic et al. 2009, 2012). Masculinized rodents have lower peripheral CD4/CD8 T-cell ratios and higher natural killer (NK) and $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T cells in peripheral blood compared with control animals. In human studies, adult females

have higher $CD4^+$ T-cell counts and greater CD4/CD8 ratios than adult males, while males may have higher $CD8^+$ T-cell counts than females (Tollerud et al. 1989; Maini et al. 1996; Uppal et al. 2003; Wong et al. 2013). This has been described in children too (Lee et al. 1996; Lisse et al. 1997). By contrast, an American study reported no sex differences in $CD4^+$ and $CD8^+$ T cells and B cells in children in the first 2 years of life (Stern et al. 1992), and a Zambian study also failed to find sex differences in $CD4^+$ and $CD8^+$ T cells in children, although a higher CD4:CD8 ratio was found for females compared with males (Ndhlovu et al. 2004).

10.3.5 Sex-Differential Effects of Breastfeeding

Multiple nutritional factors are known to influence the outcome of childhood infections, including breast versus formula feeding, weaning practices, diet, malnutrition, and micronutrient levels and supplementation. Infants that are exclusively breastfed throughout the first 6 months of life have a 14 times lower pneumonia mortality than non-breastfed infants (Lamberti et al. 2013). Human breast milk is also a good source of protective maternal antibodies for the neonate. It contains a high concentration of secretory IgA and IgG together with cytokines, antibacterial peptides, and immune cells (Brandtzaeg 2010). Secretory IgG provides protection to infants by binding pathogens and preventing them attaching to the infants' cells. Secretory IgG has the ability to survive in the respiratory and gastrointestinal mucosal membranes of infants and is not affected by proteolytic enzymes (Brandtzaeg 2010; Jackson and Nazar 2006). Breastfeeding has also been shown to modulate innate immune responses (LeBouder et al. 2006; Belderbos et al. 2012). Furthermore, exclusive breastfeeding is associated with improved thymic function (Ngom et al. 2004), and formula-fed infants have a decreased thymus size compared to breastfed infants (Hasselbalch et al. 1996).

A series of studies have addressed whether breastfeeding practices vary according to the sex of the child. One such study in an urban Hindu society in India, where a bias in infant rearing prevails toward males, showed a significantly lower risk of early weaning among male compared to female infants (Nath and Goswami 1997). Since resource abundance or scarcity are thought to effect male reproductive success more than female, natural selection should select parents who favor males when conditions are good and females when conditions are poor (Cronk 2007). The Trivers–Willard hypothesis predicts that parents in good socioeconomic conditions will bias their investment toward sons and that those in poorer condition will bias their investment toward daughters (Gaulin and Robbins 1991). A study of 900 US mothers supported this theory (Gaulin and Robbins 1991), and a Polish study found that first-born males were breastfed longer than females in highly educated families, while the opposite is true in families with the lowest education (Koziel and Ulijaszek 2001). A Kenyan study showed that while

breastfeeding frequency was equivalent in rich and poor families, the better off mothers produced richer milk (higher fat concentration) for sons than daughters (2.8 vs. 1.74 g/dl), while poorer mothers produced richer milk for daughters than sons (2.6 vs. 2.3 g/dl) (Fujita et al. 2012). Whether this phenomenon is more generalizable to other parts of the world is not known, but could account for some sex differences in immunity in early life.

Most have assumed that breast milk would confer equal protection to males and females via the passive transfer of maternal antibodies and other anti-infective factors. A case–control study conducted in Boston, USA, suggested that breastfed and mixed-fed females were at lower risk of neonatal respiratory tract infection, while for males there was no association between breastfeeding and risk (Sinha et al. 2003). This was confirmed in a subsequent prospective study of 119 very low-birth-weight infants, whereby breastfeeding protected female but not male infants against severe acute lung disease (Klein et al. 2008). The protective effect of breastfeeding persisted throughout the first year of life in females, with a decrease in episode severity but not episodes per se, and formula-fed females suffered the most severe disease. Furthermore, among breastfed children, male sex was a risk factor for diarrhea, whereas this was not the case for weaned children (Molbak et al. 1997). Together, these studies suggest an immunomodulatory effect of breast milk benefiting infant females more than males.

10.3.6 Sex Differences in Effects of Nutrition and Micronutrient Status

Micronutrient deficiency is commonplace in infancy and early childhood in many of the poorer socioeconomic areas of the world. Numerous studies have shown the benefits of multiple micronutrient supplements (MMS) such as sprinkles (Suchdev et al. 2012) or lipid-based (Iannotti et al. 2014) supplements, to supplementation with single agents such as vitamin A (Fawzi et al. 1993) and zinc (Brown et al. 2013). However, other studies show that MMS may also be detrimental, as observed in a Pakistani study in which daily supplementation of 6–18-month-old children with micronutrient powder increased severe diarrhea and respiratory infections (Soofi et al. 2013). Micronutrients are required for optimal immune functioning, and an inadequate intake leads to immunosuppression. Vitamins C and E, selenium, copper, and zinc all have antioxidant properties and thus help control tissue damage caused by reactive oxygen molecules and modulate redox-sensitive transcription factors (Wintergerst et al. 2007). Vitamins B6, folate, B12, C, and E and selenium, zinc, copper, and iron all support Th1 proinflammatory immune responses, while vitamins A and D may support a Th2 anti-inflammatory profile and have roles in cell-mediated and humoral responses (Wintergerst et al. 2007).

There is accumulating evidence that micronutrients act differently in males and females. A paper in 2009 showed that zinc, folic acid, and iron may not benefit young females, possibly due to the fact that they had recently received diphtheria, tetanus, whole-cell pertussis (DTwP) vaccine, although this is just a speculation (Benn et al. 2009). In a randomized placebo-controlled trial conducted in Tanzania, perinatal and postnatal vitamin B complex, C, and E supplementation of HIV-infected mothers was associated with decreased risk of low birth weight in neonatal females and a 32 % reduction in mortality among females, but no such effect for males (Kawai et al. 2010). A number of other studies have also shown that maternal micronutrient supplements benefit females more than males in Africa and Asia (Osrin et al. 2005; Friis et al. 2004; Fawzi et al. 2007). This indicates that sex-differential effects of nutrition commence during pregnancy. Sex differences in responses to individual micronutrient agents will be discussed below.

10.3.6.1 Sex Differences in the Effects of Vitamin A

The World Health Organization (WHO) recommends the administration of periodic high-dose vitamin A supplements to children aged 6–59 months living in low-income countries, since it has been shown to reduce all-cause mortality by 23–30 % in this age group (Glasziou and Mackerras 1993). Whether vitamin A supplementation is beneficial prior to 6 months of age remains a matter of controversy with possible benefits in Asian infants, but no benefit or even negative effects in African infants (Gogia and Sachdev 2009). This is the subject of several ongoing randomized controlled trials commissioned by the WHO. Vitamin A deficiency causes decreased mucosal epithelial integrity, suppressed innate immunity, a reduction in B and T lymphocyte numbers and possibly function, enhanced induction of $FOXP3^+$ regulatory T cells, and enhanced Th2 immunity with inhibition of Th1 immunity (Savy et al. 2009). In 43 studies of the effect of vitamin A on vaccine responses, it seemed that there was a small decrease in tuberculin skin test reactivity, a possible enhancing effect on measles, hepatitis B, and rabies antibodies, but no effect on antibodies to diphtheria, tetanus, cholera, influenza, *Haemophilus influenzae* b, or pneumococcal vaccines (Savy et al. 2009).

Sex differences in the vitamin A enhancement of immunity to certain coadministered vaccines have been described. For examples, vitamin A supplementation (VAS) alongside measles vaccine at 6 months of age increases MV antibody titers significantly in males, but not in females (Benn et al. 1997). In another study, VAS decreased the prevalence of in vitro PPD responders 2 months after supplementation of male but not female infants (Diness et al. 2007). VAS may also have a sex-differential effect on adverse reactions to vaccination, because it was associated with increased symptoms of raised intracranial pressure and decreased prevalence of fever following live vaccines in males, but neither of these effects in females (Fisker et al. 2013).

Sex-differential effects of VAS on all-cause mortality have also been described in several studies. For example, males had reduced mortality following neonatal

VAS, while females were not affected (Humphrey et al. 1996; Rahmathullah et al. 2003; Benn et al. 2008a). This may in part depend on the vitamin A dose, since a lower dose of VAS at 6 months of age reduced mortality in females but not in males when compared to standard dose (Benn et al. 2005); although a subsequent similar study of VAS at 6 months of age failed to find any overall or sex-differential effect of a lower dose VAS on child mortality (Yakymenko et al. 2011). A randomized trial of VAS at birth to low-birth-weight neonates in Guinea-Bissau showed that VAS reduced mortality in males, but increased it in females (Benn et al. 2010).

Vitamin A may also enhance the previously described heterologous effects of vaccines on susceptibility to infections and death (Benn et al. 2003; Flanagan et al. 2011, 2013). In a randomized trial of a standard dose measles vaccine (MV) at 4.5 months of age, a slightly beneficial effect of MV was reversed to a dramatic detrimental effect on all-cause mortality if the child had received VAS at birth, but only for males (Aaby et al. 2010b).

Together the above studies suggest that males are more susceptible to the immunomodulatory effects of vitamin A when administered to infants and children <5 years of age. The precise nature of these effects and underlying immunological mechanisms are currently being explored, but as yet remain unclear.

10.3.6.2 No Sex Differences in the Effects of Vitamin D

Vitamin D deficiency causes depressed innate immunity (macrophage activity), decreased lymphocyte number and function, and a shift to Th1 immunity (Savy et al. 2009). Several human studies have shown no effect of vitamin D supplementation on influenza or hepatitis B vaccine responses (Savy et al. 2009). One study found that BCG-vaccinated infants had higher vitamin D levels suggesting that BCG vaccination can lead to increased vitamin D and also that IFN-γ responses to PPD were inversely related to vitamin D levels suggesting that vitamin D may play an immunoregulatory role following BCG vaccination (Lalor et al. 2011). Sex was controlled for in this study, but no sex bias was found.

10.3.6.3 Sex Differences in the Effects of Iron

Iron deficiency causes decreased innate immunity (neutrophils, NK cells, macrophages) and decreased T lymphocyte numbers (Mullick et al. 2006). Iron supplementation improves iron deficiency and may have beneficial effects on cell-mediated immunity (Berger et al. 2000), although other studies report no benefit (Thibault et al. 1993). Intermittent iron supplementation has been recommended for preschool children up to 5 years of age in certain settings (2011). While iron supplementation may decrease iron deficiency anemia, it may also increase the risk of infection, particularly malaria, and thus the risks and benefits must be carefully weighed up (Berglund and Domellof 2014). There are limited data for

the effects of iron on vaccine responses, but of those studies available, there seemed to be no effect of iron deficiency anemia on vaccine antibody responses in humans (Savy et al. 2009). A study in Southeast Asia showed that males suffer more anemia and iron deficiency than females, suggesting that male iron requirements are higher than those of females (Wieringa et al. 2007), and this male propensity for iron deficiency anemia has been described by others (Domellof et al. 2002). This could lead to sex differences in susceptibility to infections and also in the benefits of iron supplementation.

10.3.6.4 Sex Differences in the Effects of Zinc

Zinc deficiency is very common throughout the world and causes weight loss, anorexia, poor growth, diarrhea, poor wound healing, and recurrent infections (Khalid et al. 2014). Zinc deficiency decreases innate immunity (macrophages, NK cells), causes thymic atrophy and decreased T and B lymphocyte numbers and function alongside increased apoptosis, and shifts to Th2 immunity (Keen and Gershwin 1990; Shankar and Prasad 1998). Studies of the benefits of zinc supplementation remain inconclusive, but show a clear effect on the incidence of diarrhea, and may reduce pneumonia deaths by 20 % (Penny 2013). In a zinc supplementation trial of 6–30-month-old children in Burkina Faso, clear benefits for males were observed, with protection against diarrhea, but increased ear infections compared to control males, while supplemented females had fewer ear and eye infections and better growth but more dysentery than non-supplemented females (Garenne et al. 2007). A randomized double-blind trial examined the effects of zinc supplementation on 27–50-month-old children. Supplemented males grew more than the non-supplemented male control group, whereas zinc-supplemented females did not have improved growth (Ruz et al. 1997). There were trends for males to have improved tuberculin responses and reduced rates of parasite re-infestation in this small study of 98 children, although these effects were not statistically significant.

10.3.7 Sex-Differential In Utero Effects on Immunity and Infectious Disease Susceptibility

Studies in animal models (rats, mice, sheep, and cows) and epidemiological studies in humans suggest that developmental programming in utero is different in males and females leading to differences in the local environment and sexually dimorphic outcomes including altered immune development (Aiken and Ozanne 2013). In rodents, shock, steroids, and famine have all been shown to increase blood pressure in male offspring, and famine and steroids negatively affect male renal function. Dietary effects in female animal models include an increased stress response following ethanol exposure and high salt diet and enhanced placental gene

expression and DNA hypomethylation in females as a result of a maternal high-fat diet. Developmental insults studied in humans include smoking, famine, antenatal steroid administration, asthma, and obesity. In females, famine, asthma, and steroid administration cause increased obesity, altered glucocorticoid metabolism, and increased blood pressure, respectively, while in males, smoking and alcohol intake during pregnancy cause decreased weight/head circumference and increased cortisol, respectively (Aiken and Ozanne 2013). Overall, the studies suggest greater adaptability among females in the intrauterine environment which may be an evolutionary adaptation to preserve resources during times of stress and invest in protecting females more than males and provide a reproductive advantage, since the former have more vulnerable reproductive tracts. Furthermore, males grow more quickly in utero than females (de Onis et al. 2009) and hence undergo more cell cycles during any particular insult and are thus more susceptible to adverse effects.

In humans, premature males have a 20 % reduced survival compared to females (Vatten and Skjaerven 2004), and sexual dimorphism in fetoplacental immune function may play a key role in this survival difference. The placentas from premature (<32 weeks) male neonates tend to be more chronically inflamed with increased decidual lymphoplasmacytic cell infiltration compared with those from females (Ghidini and Salafia 2005; Goldenberg et al. 2006). Furthermore, peripheral vasodilatation of the microvasculature is markedly lower in female neonates compared to males (Stark et al. 2008). This provides considerable survival benefit to females due to better cardiovascular stability and lower levels of circulating cytokines. Having said this, female fetuses were twice as likely compared with male fetuses to be vertically infected with hepatitis C virus (European Paediatric Hepatitis C Virus Network 2005).

Male fetuses are exposed to more androgens in utero than females (Barry et al. 2010) since male testes can produce androgens from 10 weeks of gestation (Carr et al. 1983), with all the subsequent immunological effects of androgen exposure described previously. Cord blood IgE is a fetal product that may predict the development of atopy, and a number of studies have shown that male neonates have higher cord blood IgE levels than females (Bergmann et al. 1995; Liu et al. 2003). Studies further suggest that women carrying a male fetus tend to have higher total IgE levels (Loken et al. 2010). Whether cord blood IgE levels have an effect on the immune response to infectious diseases or vaccination in early life is not known.

Adverse fetal conditions may cause epigenetic adaptations leading to altered gene activity that can persist throughout life (Gluckman et al. 2005; Bateson et al. 2004; Ke et al. 2006). Humans experiencing prenatal exposure to famine have clear sex differences in their DNA methylation status for a number of loci (Tobi et al. 2009). Micronutrient deficiencies including zinc, selenium, folic acid, and vitamins B6 and B12 can all lead to alterations in DNA and histone methylation (Ulrey et al. 2005). A study of epigenetic differences in Gambian infants born to mothers participating in a blinded pre- and periconception multiple micronutrient supplementation trial found that males and females had different methylation of CpG loci in both the supplemented and non-supplemented groups. This suggests

that males and females have differential developmental trajectories commencing in utero, most likely due to physiological differences at this stage (Khulan et al. 2012). More loci underwent differential methylation in males than females, and there was little overlap between the sexes in the loci that had methylation changes in the supplemented groups. The sex-specific changes were predominantly decreases in methylation. Genes involved in immunity and defense against infection were the main category affected by micronutrient deficiency. Genes associated with susceptibility to viral, bacterial, and mycobacterial infections were affected in male and female groups, with different susceptibility genes being affected according to sex. Several genes that alter susceptibility to *Plasmodium* species were also affected. Half of the supplementation-related changes observed in cord blood were present in infants at 9 months of age, indicting long-term epigenetic reprogramming in relation to nutritional deficiency and supplementation during pregnancy.

Newborn infants acquire IgG antibodies transplacentally from their mothers which provide protection against infections encountered in the first months of life, while the other immunoglobulin subclasses are unable to cross the maternal–placental interface. The maternally acquired antibodies wane over the first 6 months of life and are usually gone by 1 year of age. IgG1 isotype is the most efficiently transferred transplacentally followed by IgG4, IgG3, and IgG2 (Palmeira et al. 2012) which effects the type of diseases to which a newborn is susceptible. For example, the main antibody subclass that protects against encapsulated bacteria is IgG2, yet it is rarely transported across the placenta to the fetus, contributing to the increased early susceptibility to these pathogens.

Maternally acquired antibodies can inhibit humoral responses to infant vaccines, including live measles vaccine (Albrecht et al. 1977) and oral poliomyelitis vaccine and non-live vaccines including pertussis (Burstyn et al. 1983; Englund et al. 1995), tetanus and diphtheria toxoids (Bjorkholm et al. 1995; Claesson et al. 1989), *Haemophilus influenzae* b conjugate vaccine (Claesson et al. 1989; Daum et al. 1991), and hepatitis A vaccine (Kanra et al. 2000). The titer of maternally acquired antibodies present at the time of immunization is thought to determine the extent of their inhibition of antibody responses in neonatal mice and humans (Gans et al. 1998, 1999; Markowitz et al. 1996; Siegrist et al. 1998). However, other studies report no influence of maternally acquired antibodies on responses to the same vaccines (Gans et al. 1998, 1999; Siegrist et al. 1998), and responses may still be protective even if some inhibition occurs (Jones et al. 2014). While maternally acquired antibodies may interfere with the generation of a humoral response to vaccination, T-cell responses do not seem to be similarly affected (Siegrist 2003).

Few studies have assessed for sex differences in passively transferred maternally acquired antibodies, but one study of 500 Swiss children found no sex differences in the transfer of antibodies against MMR (Nicoara et al. 1999). Another study showed that female infants lost measles maternally acquired antibodies more quickly than males and are thus more likely to contract measles infection than males prior to vaccination (Martins et al. 2009). Thus, there may be sex differences in the rate of maternally acquired antibody decay, which would in turn lead to sex differences in infectious disease susceptibility in the first 6 months of life.

10.3.8 Behavioral Differences Between Sexes and Disease Susceptibility

The role of behavioral factors in the sex differences in disease susceptibility and outcomes and responses to vaccines warrants consideration. However, many of the sex divergent morbidities described in this chapter occur in age groups that have little sex-determined behavioral differences (Guerra-Silveira and Abad-Franch 2013). Males have more aggressive behavior than females due to higher circulating androgen levels, but this is unlikely to have an impact in infancy and early childhood. Sex differences in exposure to infections and vectors due to behavioral differences would likewise manifest later in childhood or adult life, and occupational and recreational activities that can alter disease susceptibility are unlikely to occur in the first 5 years of life. Certain disease vectors may preferentially feed on one particular sex, for example, certain *Mansonia* mosquitos prefer female to male human hosts (Gass et al. 1982).

In certain societies, males and females may be treated differently, and this could cause sex biases in susceptibility. For example, in India a male infant might be more valued than a female infant as discussed above for breastfeeding practices, and thus males might be fed and protected better leading to improved survival among males. It has also been postulated that female children are often more prone to contracting infectious diseases outside the home (index case) and hence transmitting the disease to siblings at home (secondary cases). As secondary cases experience a more intense exposure to the pathogen, they are also more likely to succumb to the infection compared to index cases. In some cultures, females are more likely to be kept at home potentially decreasing their infectious disease exposure (Aaby 2007). Increased early life father-to-son transmission and older-brother-to-younger-sibling transmission of HBV in a Melanesian population leading to increased HBV in males compared to females have been linked to cultural practices involving very early exposure of males to HBV (Langendorfer et al. 1984). It is very difficult to tease out the role that differential treatment of one sex might have in disease susceptibility patterns within certain societies, but overall behavior differences are unlikely to be a major contributor to the sex-differential effects described in this chapter.

10.4 Concluding Remarks

Throughout this chapter, we have shown multiple ways in which male and female children have different immune systems and different responses to infections and vaccination (Table 10.1). Evidence suggests that these effects begin in the pre-implantation embryo and in utero and that such early effects will set males and females on different immunological trajectories. There is a paucity of data in children <5 years of age, and more research is needed in this age group if we are

to better understand the sexual dichotomy in human responses to immune challenge. However, it seems evident that there are multiple early life sex differences in human immunity, which may one day lead to males and females being treated differently in the context of preventing and treating infections and vaccination programs.

Table 10.1 Summary of observed sex differences in immunology and infections during childhood

Subject	Sex differences in <5 years old children
Bacterial and viral infections	For most infections, an excess male incidence and mortality. The male preponderance may decrease in later childhood and for some diseases revert to excess female incidence
Parasitic infections	Male children more susceptible to most but not all parasitic infections compared to females
Immunological responses to vaccines	Females may respond with higher vaccine-specific antibody titers. No evidence of differences in cellular responses
Nontargeted effects of vaccination	Heterologous effects of vaccines may be stronger in females
Adverse events (AE) of vaccines	Although not unequivocally, males tend to experience more AE in early life, although this may switch in later childhood
Testosterone	Levels surge in males at 2–3 months of age but are equal in males and females by 12 months of age
Estradiol	Females have higher levels than males, whose levels decline more rapidly from birth to a nadir around 4 months of age
Leutinizing hormone (LH) Follicle-stimulating hormone (FSH)	Higher in males in the first 6 months, after which sex differences are leveled out Levels are higher in females throughout early childhood
Immune activation	Females have higher inflammatory responses than males (C reactive protein and erythrocyte sedimentation rate)
Innate immunity	Infant males may have more robust innate immunity than females
Thymic function/adaptive immunity	Smaller thymus size in females; higher CD4:CD8 ratio in females
Breastfeeding	Breastfeeding more beneficial to female infants than male infants with respect to protection from respiratory infections
Vitamin A supplementation (VAS)	VAS has been associated with improved survival in males but not females, or even increased mortality in females. VAS may enhance vaccine responses, heterologous effects, and AEs more in males than females
Perinatal immunology	Females generally have greater adaptability to stresses in utero. The maternal–placental interface may be more inflamed in a pregnancy with a male fetus. Male/female differences in epigenetic responses to micronutrient supplements
Passive immunity	Transfer of maternal antibodies to the fetus may be similar in both sexes, but levels may decline faster in females after birth as seen for measles antibodies

An overview of studies describing sex differences in infectious diseases and immunology in early childhood as discussed in Chap. 10. Some of the listed observations have not been unequivocally confirmed in all studies and should therefore be interpreted as tendencies

References

Aaby P (2007) Is susceptibility to severe infection in low-income countries inherited or acquired? J Intern Med 261(2):112–122. doi:10.1111/j.1365-2796.2006.01742.x

Aaby P, Samb B, Simondon F, Knudsen K, Seck AM, Bennett J, Markowitz L, Rhodes P, Whittle H (1994) Sex-specific differences in mortality after high-titre measles immunization in rural Senegal. Bull World Health Organ 72(5):761–770

Aaby P, Samb B, Simondon F, Seck AM, Knudsen K, Whittle H (1995) Non-specific beneficial effect of measles immunisation: analysis of mortality studies from developing countries. BMJ (Clinl Res Ed) 311(7003):481–485

Aaby P, Marx C, Trautner S, Rudaa D, Hasselbalch H, Jensen H, Lisse I (2002) Thymus size at birth is associated with infant mortality: a community study from Guinea-Bissau. Acta Paediatrica (Oslo, Norway: 1992) 91(6):698–703

Aaby P, Jensen H, Samb B, Cisse B, Sodemann M, Jakobsen M, Poulsen A, Rodrigues A, Lisse IM, Simondon F, Whittle H (2003) Differences in female–male mortality after high-titre measles vaccine and association with subsequent vaccination with diphtheria-tetanus-pertussis and inactivated poliovirus: reanalysis of West African studies. Lancet 361(9376): 2183–2188. doi:10.1016/s0140-6736(03)13771-3

Aaby P, Ibrahim SA, Libman MD, Jensen H (2006a) The sequence of vaccinations and increased female mortality after high-titre measles vaccine: trials from rural Sudan and Kinshasa. Vaccine 24(15):2764–2771. doi:10.1016/j.vaccine.2006.01.004

Aaby P, Jensen H, Walraven G (2006b) Age-specific changes in the female–male mortality ratio related to the pattern of vaccinations: an observational study from rural Gambia. Vaccine 24(22):4701–4708. doi:10.1016/j.vaccine.2006.03.038

Aaby P, Vessari H, Nielsen J, Maleta K, Benn CS, Jensen H, Ashorn P (2006c) Sex differential effects of routine immunizations and childhood survival in rural Malawi. Pediatr Infect Dis J 25(8):721–727. doi:10.1097/01.inf.0000227829.64686.ae

Aaby P, Benn CS, Nielsen J, Lisse IM, Rodrigues A, Jensen H (2007a) DTP vaccination and child survival in observational studies with incomplete vaccination data. Trop Med Int Health 12(1):15–24. doi:10.1111/j.1365-3156.2006.01774.x

Aaby P, Biai S, Veirum JE, Sodemann M, Lisse I, Garly ML, Ravn H, Benn CS, Rodrigues A (2007b) DTP with or after measles vaccination is associated with increased in-hospital mortality in Guinea-Bissau. Vaccine 25(7):1265–1269. doi:10.1016/j.vaccine.2006.10.007

Aaby P, Garly ML, Nielsen J, Ravn H, Martins C, Bale C, Rodrigues A, Benn CS, Lisse IM (2007c) Increased female–male mortality ratio associated with inactivated polio and diphtheria-tetanus-pertussis vaccines: observations from vaccination trials in Guinea-Bissau. Pediatr Infect Dis J 26(3):247–252

Aaby P, Martins C, Bale C, Garly ML, Rodrigues A, Biai S, Lisse IM, Whittle H, Benn CS (2010a) Sex differences in the effect of vaccines on the risk of hospitalization due to measles in Guinea-bissau. Pediatr Infect Dis J 29(4):324–328. doi:10.1097/INF.0b013e3181c15367

Aaby P, Martins CL, Garly ML, Bale C, Andersen A, Rodrigues A, Ravn H, Lisse IM, Benn CS, Whittle HC (2010b) Non-specific effects of standard measles vaccine at 4.5 and 9 months of age on childhood mortality: randomised controlled trial. BMJ (Clin Res Ed) 341:c6495. doi:10.1136/bmj.c6495

Aaby P, Roth A, Ravn H, Napirna BM, Rodrigues A, Lisse IM, Stensballe L, Diness BR, Lausch KR, Lund N, Biering-Sorensen S, Whittle H, Benn CS (2011) Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? J Infect Dis 204(2):245–252. doi:10.1093/infdis/jir240

Aaby P, Benn C, Nielsen J, Lisse IM, Rodrigues A, Ravn H (2012a) Testing the hypothesis that diphtheria-tetanus-pertussis vaccine has negative non-specific and sex-differential effects on child survival in high-mortality countries. BMJ Open 2(3). doi:10.1136/bmjopen-2011-000707

Aaby P, Ravn H, Roth A, Rodrigues A, Lisse IM, Diness BR, Lausch KR, Lund N, Rasmussen J, Biering-Sorensen S, Whittle H, Benn CS (2012b) Early diphtheria-tetanus-pertussis

vaccination associated with higher female mortality and no difference in male mortality in a cohort of low birthweight children: an observational study within a randomised trial. Arch Dis Child 97(8):685–691. doi:10.1136/archdischild-2011-300646

Agergaard J, Nante E, Poulstrup G, Nielsen J, Flanagan KL, Ostergaard L, Benn CS, Aaby P (2011) Diphtheria-tetanus-pertussis vaccine administered simultaneously with measles vaccine is associated with increased morbidity and poor growth in girls. A randomised trial from Guinea-Bissau. Vaccine 29(3):487–500. doi:10.1016/j.vaccine.2010.10.071

Aiken CE, Ozanne SE (2013) Sex differences in developmental programming models. Reproduction 145:R1–R13

Aksoy E, Albarani V, Nguyen M, Laes JF, Ruelle JL, De Wit D, Willems F, Goldman M, Goriely S (2007) Interferon regulatory factor 3-dependent responses to lipopolysaccharide are selectively blunted in cord blood cells. Blood 109(7):2887–2893. doi:10.1182/blood-2006-06-027862

Albers MJ, de Gast-Bakker DA, van Dam NA, Madern GC, Tibboel D (2002) Male sex predisposes the newborn surgical patient to parenteral nutrition-associated cholestasis and to sepsis. Arch Surg (Chicago, IL : 1960) 137(7):789–793

Albrecht P, Ennis FA, Saltzman EJ, Krugman S (1977) Persistence of maternal antibody in infants beyond 12 months: mechanism of measles vaccine failure. J Pediatr 91(5):715–718

Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O, Skakkebaek NE (1998) Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. J Clin Endocrinol Metabol 83(2):675–681. doi:10.1210/jcem.83.2.4603

Angele MK, Schwacha MG, Ayala A, Chaudry IH (2000) Effect of gender and sex hormones on immune responses following shock. Shock (Augusta, GA) 14(2):81–90

Angelone DF, Wessels MR, Coughlin M, Suter EE, Valentini P, Kalish LA, Levy O (2006) Innate immunity of the human newborn is polarized toward a high ratio of IL-6/TNF-alpha production in vitro and in vivo. Pediatr Res 60(2):205–209. doi:10.1203/01.pdr.0000228319.10481.ea

Atabani S, Landucci G, Steward MW, Whittle H, Tilles JG, Forthal DN (2000) Sex-associated differences in the antibody-dependent cellular cytotoxicity antibody response to measles vaccines. Clin Diagn Lab Immunol 7(1):111–113

Baker JP (2011) The first measles vaccine. Pediatrics 128(3):435–437. doi:10.1542/peds.2011-1430

Barry JA, Kay AR, Navaratnarajah R, Iqbal S, Bamfo JE, David AL, Hines M, Hardiman PJ (2010) Umbilical vein testosterone in female infants born to mothers with polycystic ovary syndrome is elevated to male levels. J Obstet Gynaecol 30(5):444–446. doi:10.3109/01443615.2010.485254

Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, Gluckman P, Godfrey K, Kirkwood T, Lahr MM, McNamara J, Metcalfe NB, Monaghan P, Spencer HG, Sultan SE (2004) Developmental plasticity and human health. Nature 430(6998):419–421. doi:10.1038/nature02725

Belderbos ME, van Bleek GM, Levy O, Blanken MO, Houben ML, Schuijff L, Kimpen JL, Bont L (2009) Skewed pattern of toll-like receptor 4-mediated cytokine production in human neonatal blood: low LPS-induced IL-12p70 and high IL-10 persist throughout the first month of life. Clin Immunol 133(2):228–237. doi:10.1016/j.clim.2009.07.003

Belderbos ME, Houben ML, van Bleek GM, Schuijff L, van Uden NO, Bloemen-Carlier EM, Kimpen JL, Eijkemans MJ, Rovers M, Bont LJ (2012) Breastfeeding modulates neonatal innate immune responses: a prospective birth cohort study. Pediatr Allerg Immunol 23(1):65–74. doi:10.1111/j.1399-3038.2011.01230.x

Bellamy GJ, Hinchliffe RF, Crawshaw KC, Finn A, Bell F (2000) Total and differential leucocyte counts in infants at 2, 5 and 13 months of age. Clin Lab Haematol 22(2):81–87

Benn CS, Aaby P, Bale C, Olsen J, Michaelsen KF, George E, Whittle H (1997) Randomised trial of effect of vitamin A supplementation on antibody response to measles vaccine in Guinea-Bissau, west Africa. Lancet 350(9071):101–105. doi:10.1016/s0140-6736(96)12019-5

Benn CS, Bale C, Sommerfelt H, Friis H, Aaby P (2003) Hypothesis: vitamin A supplementation and childhood mortality: amplification of the non-specific effects of vaccines? Int J Epidemiol 32(5):822–828

Benn CS, Martins C, Rodrigues A, Jensen H, Lisse IM, Aaby P (2005) Randomised study of effect of different doses of vitamin A on childhood morbidity and mortality. BMJ (Clin Res Ed) 331 (7530):1428–1432. doi:10.1136/bmj.38670.639340.55

Benn CS, Diness BR, Roth A, Nante E, Fisker AB, Lisse IM, Yazdanbakhsh M, Whittle H, Rodrigues A, Aaby P (2008a) Effect of 50,000 IU vitamin A given with BCG vaccine on mortality in infants in Guinea-Bissau: randomised placebo controlled trial. BMJ (Clin Res Ed) 336(7658):1416–1420. doi:10.1136/bmj.39542.509444.AE

Benn CS, Fisker AB, Rodrigues A, Ravn H, Sartono E, Whittle H, Yazdanbakhsh M, Aaby P (2008b) Sex-differential effect on infant mortality of oral polio vaccine administered with BCG at birth in Guinea-Bissau. A natural experiment. PLoS ONE 3(12):e4056. doi:10.1371/journal.pone.0004056

Benn CS, Lund S, Fisker A, Jorgensen MJ, Aaby P (2009) Should infant girls receive micronutrient supplements? Int J Epidemiol 38(2):586–590. doi:10.1093/ije/dyn364

Benn CS, Fisker AB, Napirna BM, Roth A, Diness BR, Lausch KR, Ravn H, Yazdanbakhsh M, Rodrigues A, Whittle H, Aaby P (2010) Vitamin A supplementation and BCG vaccination at birth in low birthweight neonates: two by two factorial randomised controlled trial. BMJ (Cli Res Ed) 340:c1101. doi:10.1136/bmj.c1101

Berger J, Dyck JL, Galan P, Aplogan A, Schneider D, Traissac P, Hercberg S (2000) Effect of daily iron supplementation on iron status, cell-mediated immunity, and incidence of infections in 6–36 month old Togolese children. Eur J Clin Nutr 54(1):29–35

Berglund S, Domellof M (2014) Meeting iron needs for infants and children. Curr Opin Clin Nutr Metabol Care. doi:10.1097/mco.0000000000000043

Bergmann RL, Schulz J, Gunther S, Dudenhausen JW, Bergmann KE, Bauer CP, Dorsch W, Schmidt E, Luck W, Lau S et al (1995) Determinants of cord-blood IgE concentrations in 6401 German neonates. Allergy 50(1):65–71

Beverley JK, Fleck DG, Kwantes W, Ludlam GB (1976) Age-sex distribution of various diseases with particular reference to toxoplasmic lymphadenopathy. J Hyg 76(2):215–228

Biering-Sorensen S, Aaby P, Napirna BM, Roth A, Ravn H, Rodrigues A, Whittle H, Benn CS (2012) Small randomized trial among low-birth-weight children receiving bacillus Calmette-Guerin vaccination at first health center contact. Pediatr Infect Dis J 31(3):306–308. doi:10.1097/INF.0b013e3182458289

Bjorkholm B, Granstrom M, Taranger J, Wahl M, Hagberg L (1995) Influence of high titers of maternal antibody on the serologic response of infants to diphtheria vaccination at three, five and twelve months of age. Pediatr Infect Dis J 14(10):846–850

Brandtzaeg P (2010) The mucosal immune system and its integration with the mammary glands. J Pediatr 156(2 Suppl):S8–S15. doi:10.1016/j.jpeds.2009.11.014

Brown KH, Hess SY, Vosti SA, Baker SK (2013) Comparison of the estimated cost-effectiveness of preventive and therapeutic zinc supplementation strategies for reducing child morbidity and mortality in sub-Saharan Africa. Food Nutr Bull 34(2):199–214

Burl S, Adetifa UJ, Cox M, Touray E, Ota MO, Marchant A, Whittle H, McShane H, Rowland-Jones SL, Flanagan KL (2010) Delaying bacillus Calmette-Guerin vaccination from birth to 4 1/2 months of age reduces postvaccination Th1 and IL-17 responses but leads to comparable mycobacterial responses at 9 months of age. J Immunol 185(4):2620–2628. doi:10.4049/jimmunol.1000552

Burl S, Townend J, Njie-Jobe J, Cox M, Adetifa UJ, Touray E, Philbin VJ, Mancuso C, Kampmann B, Whittle H, Jaye A, Flanagan KL, Levy O (2011) Age-dependent maturation of Toll-like receptor-mediated cytokine responses in Gambian infants. PLoS ONE 6(4): e18185. doi:10.1371/journal.pone.0018185

Burstyn DG, Baraff LJ, Peppler MS, Leake RD, St Geme J Jr, Manclark CR (1983) Serological response to filamentous hemagglutinin and lymphocytosis-promoting toxin of Bordetella pertussis. Infect Immun 41(3):1150–1156

Carr R (2000) Neutrophil production and function in newborn infants. Br J Haematol 110(1): 18–28

Carr BR, Parker CR Jr, Ohashi M, MacDonald PC, Simpson ER (1983) Regulation of human fetal testicular secretion of testosterone: low-density lipoprotein-cholesterol and cholesterol synthesized de novo as steroid precursor. Am J Obstet Gynecol 146(3):241–247

Carrasco-Garrido P, Gallardo-Pino C, Jimenez-Garcia R, Tapias MA, de Miguel AG (2004) Incidence of adverse reactions to vaccines in a paediatric population. Clin Drug Invest 24(8): 457–463

Casimir GJ, Heldenbergh F, Hanssens L, Mulier S, Heinrichs C, Lefevre N, Desir J, Corazza F, Duchateau J (2010a) Gender differences and inflammation: an in vitro model of blood cells stimulation in prepubescent children. J Inflamm (London, England) 7:28. doi:10.1186/1476-9255-7-28

Casimir GJ, Mulier S, Hanssens L, Zylberberg K, Duchateau J (2010b) Gender differences in inflammatory markers in children. Shock (Augusta, GA) 33(3):258–262. doi:10.1097/SHK.0b013e3181b2b36b

Chan GJ, Moulton LH, Becker S, Munoz A, Black RE (2007) Non-specific effects of diphtheria tetanus pertussis vaccination on child mortality in Cebu, The Philippines. Int J Epidemiol 36(5):1022–1029. doi:10.1093/ije/dym142

Chen HL, Chang CJ, Kong MS, Huang FC, Lee HC, Lin CC, Liu CC, Lee IH, Wu TC, Wu SF, Ni YH, Hsu HY, Chen DS, Chang MH (2004) Pediatric fulminant hepatic failure in endemic areas of hepatitis B infection: 15 years after universal hepatitis B vaccination. Hepatology (Baltimore, MD) 39(1):58–63. doi:10.1002/hep.20006

Choudhry MA, Bland KI, Chaudry IH (2007) Trauma and immune response–effect of gender differences. Injury 38(12):1382–1391. doi:10.1016/j.injury.2007.09.027

Christy C, Pichichero ME, Reed GF, Decker MD, Anderson EL, Rennels MB, Englund JA, Edwards KM, Steinhoff MC (1995) Effect of gender, race, and parental education on immunogenicity and reported reactogenicity of acellular and whole-cell pertussis vaccines. Pediatrics 96(3 Pt 2):584–587

Claesson BA, Schneerson R, Robbins JB, Johansson J, Lagergard T, Taranger J, Bryla D, Levi L, Cramton T, Trollfors B (1989) Protective levels of serum antibodies stimulated in infants by two injections of Haemophilus influenzae type b capsular polysaccharide-tetanus toxoid conjugate. J Pediatr 114(1):97–100

Cook IF (2008) Sexual dimorphism of humoral immunity with human vaccines. Vaccine 26(29–30):3551–3555. doi:10.1016/j.vaccine.2008.04.054

Corbett NP, Blimkie D, Ho KC, Cai B, Sutherland DP, Kallos A, Crabtree J, Rein-Weston A, Lavoie PM, Turvey SE, Hawkins NR, Self SG, Wilson CB, Hajjar AM, Fortuno ES 3rd, Kollmann TR (2010) Ontogeny of Toll-like receptor mediated cytokine responses of human blood mononuclear cells. PLoS ONE 5(11):e15041. doi:10.1371/journal.pone.0015041

Cronk L (2007) Boy or girl: gender preferences from a Darwinian point of view. Reprod Biomed Online 15(Suppl 2):23–32

Danis B, George TC, Goriely S, Dutta B, Renneson J, Gatto L, Fitzgerald-Bocarsly P, Marchant A, Goldman M, Willems F, De Wit D (2008) Interferon regulatory factor 7-mediated responses are defective in cord blood plasmacytoid dendritic cells. Eur J Immunol 38(2):507–517. doi:10.1002/eji.200737760

Daum RS, Siber GR, Ballanco GA, Sood SK (1991) Serum anticapsular antibody response in the first week after immunization of adults and infants with the Haemophilus influenzae type b-Neisseria meningitidis outer membrane protein complex conjugate vaccine. J Infect Dis 164(6):1154–1159

de Onis M, Garza C, Onyango AW, Rolland-Cachera MF (2009) WHO growth standards for infants and young children. Archives de Pediatrie 16(1):47–53. doi:10.1016/j.arcped.2008.10.010

De Wit D, Olislagers V, Goriely S, Vermeulen F, Wagner H, Goldman M, Willems F (2004) Blood plasmacytoid dendritic cell responses to CpG oligodeoxynucleotides are impaired in human newborns. Blood 103(3):1030–1032. doi:10.1182/blood-2003-04-1216

Degu G, Mengistu G, Jones J (2002) Some factors affecting prevalence of and immune responses to Schistosoma mansoni in schoolchildren in Gorgora, northwest Ethiopia. Ethiop Med J 40(4):345–352

Dhiman N, Ovsyannikova IG, Ryan JE, Jacobson RM, Vierkant RA, Pankratz VS, Jacobsen SJ, Poland GA (2005) Correlations among measles virus-specific antibody, lymphoproliferation and Th1/Th2 cytokine responses following measles-mumps-rubella-II (MMR-II) vaccination. Clin Exp Immunol 142(3):498–504. doi:10.1111/j.1365-2249.2005.02931.x

Diness BR, Fisker AB, Roth A, Yazdanbakhsh M, Sartono E, Whittle H, Nante JE, Lisse IM, Ravn H, Rodrigues A, Aaby P, Benn CS (2007) Effect of high-dose vitamin A supplementation on the immune response to Bacille Calmette-Guerin vaccine. Am J Clin Nutr 86(4):1152–1159

Domellof M, Lonnerdal B, Dewey KG, Cohen RJ, Rivera LL, Hernell O (2002) Sex differences in iron status during infancy. Pediatrics 110(3):545–552

Drevenstedt GL, Crimmins EM, Vasunilashorn S, Finch CE (2008) The rise and fall of excess male infant mortality. Proc Natl Acad Sci USA 105(13):5016–5021. doi:10.1073/pnas.0800221105

Englund JA, Anderson EL, Reed GF, Decker MD, Edwards KM, Pichichero ME, Steinhoff MC, Rennels MB, Deforest A, Meade BD (1995) The effect of maternal antibody on the serologic response and the incidence of adverse reactions after primary immunization with acellular and whole-cell pertussis vaccines combined with diphtheria and tetanus toxoids. Pediatrics 96(3 Pt 2):580–584

Eshima N, Tokumaru O, Hara S, Bacal K, Korematsu S, Karukaya S, Uruma K, Okabe N, Matsuishi T (2012) Age-specific sex-related differences in infections: a statistical analysis of national surveillance data in Japan. PLoS ONE 7(7):e42261. doi:10.1371/journal.pone.0042261

European Paediatric Hepatitis C Virus Network (2005) A significant sex--but not elective cesarean section--effect on mother-to-child transmission of hepatitis C virus infection. J Infect Dis 192(11):1872–1879. doi:10.1086/497695

Fang JW, Lai CL, Chung HT, Wu PC, Lau JY (1994) Female children respond to recombinant hepatitis B vaccine with a higher titre than male. J Trop Pediatr 40(2):104–107

Fawzi WW, Chalmers TC, Herrera MG, Mosteller F (1993) Vitamin A supplementation and child mortality. A meta-analysis. JAMA 269(7):898–903

Fawzi WW, Msamanga GI, Urassa W, Hertzmark E, Petraro P, Willett WC, Spiegelman D (2007) Vitamins and perinatal outcomes among HIV-negative women in Tanzania. N Engl J Med 356(14):1423–1431. doi:10.1056/NEJMoa064868

Ferguson JF, Patel PN, Shah RY, Mulvey CK, Gadi R, Nijjar PS, Usman HM, Mehta NN, Shah R, Master SR, Propert KJ, Reilly MP (2013) Race and gender variation in response to evoked inflammation. J Transl Med 11:63. doi:10.1186/1479-5876-11-63

Finan C, Ota MO, Marchant A, Newport MJ (2008) Natural variation in immune responses to neonatal Mycobacterium bovis Bacillus Calmette-Guerin (BCG) Vaccination in a Cohort of Gambian infants. PLoS ONE 3(10):e3485. doi:10.1371/journal.pone.0003485

Fine PE, Smith PG (2007) 'Non-specific effects of vaccines'–an important analytical insight, and call for a workshop. Trop Med Int Health 12(1):1–4. doi:10.1111/j.1365-3156.2006.01794.x

Fisker AB, Bale C, Jorgensen MJ, Balde I, Hornshoj L, Bibby BM, Aaby P, Benn CS (2013) High-dose vitamin A supplementation administered with vaccinations after 6 months of age: sex-differential adverse reactions and morbidity. Vaccine 31(31):3191–3198. doi:10.1016/j.vaccine.2013.04.072

Flanagan KL (2014) Sexual dimorphism in biomedical research: a call to analyse by sex. Trans R Soc Trop Med Hyg 108(7):385–387. doi:10.1093/trstmh/tru079

Flanagan KL, Klein SL, Skakkebaek NE, Marriott I, Marchant A, Selin L, Fish EN, Prentice AM, Whittle H, Benn CS, Aaby P (2011) Sex differences in the vaccine-specific and non-targeted effects of vaccines. Vaccine 29(13):2349–2354. doi:10.1016/j.vaccine.2011.01.071

Flanagan KL, van Crevel R, Curtis N, Shann F, Levy O, Optimmunize N (2013) Heterologous ("nonspecific") and sex-differential effects of vaccines: epidemiology, clinical trials, and emerging immunologic mechanisms. Clin Infect Dis 57(2):283–289. doi:10.1093/cid/cit209

Fleming DM, Cross KW, Cobb WA, Chapman RS (2004) Gender difference in the incidence of shingles. Epidemiol Infect 132(1):1–5

Forest MG, Cathiard AM, Bertrand JA (1973) Evidence of testicular activity in early infancy. J Clin Endocrinol Metabol 37(1):148–151. doi:10.1210/jcem-37-1-148

Friis H, Gomo E, Nyazema N, Ndhlovu P, Krarup H, Kaestel P, Michaelsen KF (2004) Effect of multimicronutrient supplementation on gestational length and birth size: a randomized, placebo-controlled, double-blind effectiveness trial in Zimbabwe. Am J Clin Nutr 80(1): 178–184

Fujita M, Roth E, Lo YJ, Hurst C, Vollner J, Kendell A (2012) In poor families, mothers' milk is richer for daughters than sons: a test of Trivers-Willard hypothesis in agropastoral settlements in Northern Kenya. Am J Phys Anthropol 149(1):52–59. doi:10.1002/ajpa.22092

Furman D, Hejblum BP, Simon N, Jojic V, Dekker CL, Thiebaut R, Tibshirani RJ, Davis MM (2014) Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. Proc Natl Acad Sci USA 111(2):869–874. doi:10.1073/pnas.1321060111

Gans HA, Arvin AM, Galinus J, Logan L, DeHovitz R, Maldonado Y (1998) Deficiency of the humoral immune response to measles vaccine in infants immunized at age 6 months. JAMA 280(6):527–532

Gans HA, Maldonado Y, Yasukawa LL, Beeler J, Audet S, Rinki MM, DeHovitz R, Arvin AM (1999) IL-12, IFN-gamma, and T cell proliferation to measles in immunized infants. J Immunol 162(9):5569–5575

Garenne M, Becher H, Ye Y, Kouyate B, Muller O (2007) Sex-specific responses to zinc supplementation in Nouna, Burkina Faso. J Pediatr Gastroenterol Nutr 44(5):619–628. doi:10.1097/MPG.0b013e31802c695e

Garly ML, Jensen H, Martins CL, Bale C, Balde MA, Lisse IM, Aaby P (2004) Hepatitis B vaccination associated with higher female than male mortality in Guinea-bissau: an observational study. Pediatr Infect Dis J 23(12):1086–1092

Garly ML, Trautner SL, Marx C, Danebod K, Nielsen J, Ravn H, Martins CL, Bale C, Aaby P, Lisse IM (2008) Thymus size at 6 months of age and subsequent child mortality. J Pediatr 153(5):683–688, 10.1016/j.jpeds.2008.04.069

Gass RF, Deesin T, Surathin K, Vutikes S, Sucharit S (1982) Observations on the feeding habits of four species of Mansonia (Mansonioides) mosquitoes in Southern Thailand. Southe Asian J Trop Med Pub Health 13(2):211–215

Gaulin SJ, Robbins CJ (1991) Trivers-Willard effect in contemporary North American society. Am J Phys Anthropol 85(1):61–69. doi:10.1002/ajpa.1330850108

Ghidini A, Salafia CM (2005) Histologic placental lesions in women with recurrent preterm delivery. Acta Obstet Gynecol Scand 84(6):547–550. doi:10.1111/j.0001-6349.2005.00694.x

Ghuman AK, Newth CJ, Khemani RG (2013) Impact of gender on sepsis mortality and severity of illness for prepubertal and postpubertal children. J Pediatr 163(3):835–840. doi:10.1016/j.jpeds.2013.04.018, e831

Giannoni E, Guignard L, Knaup Reymond M, Perreau M, Roth-Kleiner M, Calandra T, Roger T (2011) Estradiol and progesterone strongly inhibit the innate immune response of mononuclear cells in newborns. Infect Immun 79(7):2690–2698. doi:10.1128/IAI. 00076-11

Glasziou PP, Mackerras DE (1993) Vitamin A supplementation in infectious diseases: a meta-analysis. BMJ (Clin Res Ed) 306(6874):366–370

Gluckman PD, Hanson MA, Spencer HG, Bateson P (2005) Environmental influences during development and their later consequences for health and disease: implications for the interpretation of empirical studies. Proc Biol Sci 272(1564):671–677. doi:10.1098/rspb.2004.3001

Gogia S, Sachdev HS (2009) Neonatal vitamin A supplementation for prevention of mortality and morbidity in infancy: systematic review of randomised controlled trials. BMJ (Clin Res Ed) 338:b919. doi:10.1136/bmj.b919

Goldenberg RL, Andrews WW, Faye-Petersen OM, Goepfert AR, Cliver SP, Hauth JC (2006) The Alabama Preterm Birth Study: intrauterine infection and placental histologic findings in preterm births of males and females less than 32 weeks. Am J Obstet Gynecol 195(6): 1533–1537. doi:10.1016/j.ajog.2006.05.023

Goriely S, Van Lint C, Dadkhah R, Libin M, De Wit D, Demonte D, Willems F, Goldman M (2004) A defect in nucleosome remodeling prevents IL-12(p35) gene transcription in neonatal dendritic cells. J Exp Med 199(7):1011–1016. doi:10.1084/jem.20031272

Green MS (1992) The male predominance in the incidence of infectious diseases in children: a postulated explanation for disparities in the literature. Int J Epidemiol 21(2):381–386

Green MS, Shohat T, Lerman Y, Cohen D, Slepon R, Duvdevani P, Varsano N, Dagan R, Mendelson E (1994) Sex differences in the humoral antibody response to live measles vaccine in young adults. Int J Epidemiol 23(5):1078–1081

Guerra-Silveira F, Abad-Franch F (2013) Sex bias in infectious disease epidemiology: patterns and processes. PLoS ONE 8(4):e62390. doi:10.1371/journal.pone.0062390

Guilmot A, Hermann E, Braud VM, Carlier Y, Truyens C (2011) Natural killer cell responses to infections in early life. J Innate Immun 3(3):280–288. doi:10.1159/000323934

Haralambieva IH, Ovsyannikova IG, Kennedy RB, Larrabee BR, Shane Pankratz V, Poland GA (2013) Race and sex-based differences in cytokine immune responses to smallpox vaccine in healthy individuals. Hum Immunol 74(10):1263–1266. doi:10.1016/j.humimm.2013.06.031

Hartman ME, Linde-Zwirble WT, Angus DC, Watson RS (2013) Trends in the epidemiology of pediatric severe sepsis*. Pediatr Crit Care Med 14(7):686–693. doi:10.1097/PCC.0b013e3182917fad

Hasselbalch H, Jeppesen DL, Engelmann MD, Michaelsen KF, Nielsen MB (1996) Decreased thymus size in formula-fed infants compared with breastfed infants. Acta Paediatrica (Oslo, Norway: 1992) 85(9):1029–1032

Hirve S, Bavdekar A, Juvekar S, Benn CS, Nielsen J, Aaby P (2012) Non-specific and sex-differential effects of vaccinations on child survival in rural western India. Vaccine 30(50):7300–7308. doi:10.1016/j.vaccine.2012.09.035

Holt EA, Moulton LH, Siberry GK, Halsey NA (1993) Differential mortality by measles vaccine titer and sex. J Infect Dis 168(5):1087–1096

Humphrey JH, Agoestina T, Wu L, Usman A, Nurachim M, Subardja D, Hidayat S, Tielsch J, West KP Jr, Sommer A (1996) Impact of neonatal vitamin A supplementation on infant morbidity and mortality. J Pediatr 128(4):489–496

Hussain H, Akram DS, Chandir S, Khan AJ, Memon A, Halsey NA (2013) Immune response to 1 and 2 dose regimens of measles vaccine in Pakistani children. Hum Vacc Immunotherap 9(12):2529–2532. doi:10.4161/hv.25993

Hussey GD, Goddard EA, Hughes J, Ryon JJ, Kerran M, Carelse E, Strebel PM, Markowitz LE, Moodie J, Barron P, Latief Z, Sayed R, Beatty D, Griffin DE (1996) The effect of Edmonston-Zagreb and Schwarz measles vaccines on immune response in infants. J Infect Dis 173(6): 1320–1326

Hviid A, Melbye M (2007) The epidemiology of viral meningitis hospitalization in childhood. Epidemiology (Cambridge, MA) 18(6):695–701

Iannotti LL, Dulience SJ, Green J, Joseph S, Francois J, Antenor ML, Lesorogol C, Mounce J, Nickerson NM (2014) Linear growth increased in young children in an urban slum of Haiti: a randomized controlled trial of a lipid-based nutrient supplement. Am J Clin Nutr 99(1): 198–208. doi:10.3945/ajcn.113.063883

Ikegami S, Moriwake T, Tanaka H, Inoue M, Kubo T, Suzuki S, Kanzakili S, Seino Y (2001) An ultrasensitive assay revealed age-related changes in serum oestradiol at low concentrations in both sexes from infancy to puberty. Clin Endocrinol 55(6):789–795

Jackson KM, Nazar AM (2006) Breastfeeding, the immune response, and long-term health. J Am Osteopath Assoc 106(4):203–207

Jensen H, Benn CS, Lisse IM, Rodrigues A, Andersen PK, Aaby P (2007) Survival bias in observational studies of the impact of routine immunizations on childhood survival. Trop Med Int Health 12(1):5–14. doi:10.1111/j.1365-3156.2006.01773.x

Jensen KJ, Sondergaard M, Andersen A, Sartono E, Martins C, Garly ML, Eugen-Olsen J, Ullum H, Yazdanbakhsh M, Aaby P, Benn CS, Erikstrup C (2014) A randomized trial of an early measles vaccine at 4(1/2) months of age in Guinea-bissau: sex-differential immunological effects. PLoS ONE 9(5):e97536. doi:10.1371/journal.pone.0097536

Jensen-Fangel S, Mohey R, Johnsen SP, Andersen PL, Sorensen HT, Ostergaard L (2004) Gender differences in hospitalization rates for respiratory tract infections in Danish youth. Scand J Infect Dis 36(1):31–36

Ji C, Huang XW, Yang RW, Wang X, Zhao ZY (2008) Gonadotropins and sex hormones in healthy Chinese infants. Indian Pediatr 45(6):489–492

Jones C, Pollock L, Barnett SM, Battersby A, Kampmann B (2014) The relationship between concentration of specific antibody at birth and subsequent response to primary immunization. Vaccine 32(8):996–1002. doi:10.1016/j.vaccine.2013.11.104

Kanra G, Yalcin SS, Ceyhan M, Yurdakok K (2000) Clinical trial to evaluate immunogenicity and safety of inactivated hepatitis A vaccination starting at 2-month-old children. Turk J Pediatr 42(2):105–108

Kawai K, Msamanga G, Manji K, Villamor E, Bosch RJ, Hertzmark E, Fawzi WW (2010) Sex differences in the effects of maternal vitamin supplements on mortality and morbidity among children born to HIV-infected women in Tanzania. Br J Nutr 103(12):1784–1791. doi:10.1017/s0007114509993862

Ke X, Lei Q, James SJ, Kelleher SL, Melnyk S, Jernigan S, Yu X, Wang L, Callaway CW, Gill G, Chan GM, Albertine KH, McKnight RA, Lane RH (2006) Uteroplacental insufficiency affects epigenetic determinants of chromatin structure in brains of neonatal and juvenile IUGR rats. Physiol Genomics 25(1):16–28. doi:10.1152/physiolgenomics.00093.2005

Keen CL, Gershwin ME (1990) Zinc deficiency and immune function. Annu Rev Nutr 10: 415–431. doi:10.1146/annurev.nu.10.070190.002215

Khalid N, Ahmed A, Bhatti MS, Randhawa MA, Ahmad A, Rafaqat R (2014) A question mark on zinc deficiency in 185 million people in Pakistan-possible way out. Crit Rev Food Sci Nutr 54(9):1222–1240. doi:10.1080/10408398.2011.630541

Khulan B, Cooper WN, Skinner BM, Bauer J, Owens S, Prentice AM, Belteki G, Constancia M, Dunger D, Affara NA (2012) Periconceptional maternal micronutrient supplementation is associated with widespread gender related changes in the epigenome: a study of a unique resource in the Gambia. Hum Mol Genet 21(9):2086–2101. doi:10.1093/hmg/dds026

Klein SL (2004) Hormonal and immunological mechanisms mediating sex differences in parasite infection. Parasite Immunol 26(6–7):247–264. doi:10.1111/j.0141-9838.2004.00710.x

Klein SL, Poland GA (2013) Personalized vaccinology: one size and dose might not fit both sexes. Vaccine 31(23):2599–2600. doi:10.1016/j.vaccine.2013.02.070

Klein MI, Bergel E, Gibbons L, Coviello S, Bauer G, Benitez A, Serra ME, Delgado MF, Melendi GA, Rodriguez S, Kleeberger SR, Polack FP (2008) Differential gender response to respiratory infections and to the protective effect of breast milk in preterm infants. Pediatrics 121(6):e1510–e1516. doi:10.1542/peds. 2007-1757

Kollmann TR, Crabtree J, Rein-Weston A, Blimkie D, Thommai F, Wang XY, Lavoie PM, Furlong J, Fortuno ES 3rd, Hajjar AM, Hawkins NR, Self SG, Wilson CB (2009) Neonatal innate TLR-mediated responses are distinct from those of adults. J Immunol 183(11):7150–7160. doi:10.4049/jimmunol.0901481

Kollmann TR, Levy O, Montgomery RR, Goriely S (2012) Innate immune function by Toll-like receptors: distinct responses in newborns and the elderly. Immunity 37(5):771–783. doi:10.1016/j.immuni.2012.10.014

Koziel S, Ulijaszek SJ (2001) Waiting for Trivers and Willard: do the rich really favor sons? Am J Phys Anthropol 115(1):71–79. doi:10.1002/ajpa.1058

Lalor MK, Floyd S, Gorak-Stolinska P, Weir RE, Blitz R, Branson K, Fine PE, Dockrell HM (2011) BCG vaccination: a role for vitamin D? PLoS ONE 6(1):e16709. doi:10.1371/journal.pone.0016709

Lamberti LM, Zakarija-Grkovic I, Fischer Walker CL, Theodoratou E, Nair H, Campbell H, Black RE (2013) Breastfeeding for reducing the risk of pneumonia morbidity and mortality in children under two: a systematic literature review and meta-analysis. BMC Public Health 13(Suppl 3):S18. doi:10.1186/1471-2458-13-s3-s18

Landgraf B, Kollaritsch H, Wiedermann G, Wernsdorfer WH (1994) Parasite density of Plasmodium falciparum malaria in Ghanaian schoolchildren: evidence for influence of sex hormones? Trans R Soc Trop Med Hyg 88(1):73–74

Langendorfer A, Davenport W, London WT, Blumberg BS, Mazzur S (1984) Sex-related differences in transmission of hepatitis B infection in a Melanesian population. Am J Phys Anthropol 64(3):243–254. doi:10.1002/ajpa.1330640306

Lary JM, Paulozzi LJ (2001) Sex differences in the prevalence of human birth defects: a population-based study. Teratology 64(5):237–251. doi:10.1002/tera.1070

LeBouder E, Rey-Nores JE, Raby AC, Affolter M, Vidal K, Thornton CA, Labeta MO (2006) Modulation of neonatal microbial recognition: TLR-mediated innate immune responses are specifically and differentially modulated by human milk. J Immunol 176(6):3742–3752

Lee BW, Yap HK, Chew FT, Quah TC, Prabhakaran K, Chan GS, Wong SC, Seah CC (1996) Age- and sex-related changes in lymphocyte subpopulations of healthy Asian subjects: from birth to adulthood. Cytometry 26(1):8–15. doi:10.1002/(sici)1097-0320(19960315)26:1<8::aid-cyto2>3.0.co;2-e

Lee KY, Lee HS, Hur JK, Kang JH, Lee BC (2007) The changing epidemiology of hospitalized pediatric patients in three measles outbreaks. J Infect 54(2):167–172. doi:10.1016/j.jinf.2006.02.016

Leposavic G, Karapetrovic B, Obradovic S, Vidiic Dandovic B, Kosec D (1996) Differential effects of gonadectomy on the thymocyte phenotypic profile in male and female rats. Pharmacol Biochem Behav 54(1):269–276

Leposavic G, Perisic M, Kosec D, Arsenovic-Ranin N, Radojevic K, Stojic-Vukanic Z, Pilipovic I (2009) Neonatal testosterone imprinting affects thymus development and leads to phenotypic rejuvenation and masculinization of the peripheral blood T-cell compartment in adult female rats. Brain Behav Immun 23(2):294–304. doi:10.1016/j.bbi.2008.11.002

Leposavic G, Perisic M, Pilipovic I (2012) Role of gonadal hormones in programming developmental changes in thymopoietic efficiency and sexual diergism in thymopoiesis. Immunol Res 52(1–2):7–19. doi:10.1007/s12026-012-8278-6

Levy O (2007) Innate immunity of the newborn: basic mechanisms and clinical correlates. Nat Rev Immunol 7(5):379–390. doi:10.1038/nri2075

Levy O, Zarember KA, Roy RM, Cywes C, Godowski PJ, Wessels MR (2004) Selective impairment of TLR-mediated innate immunity in human newborns: neonatal blood plasma reduces monocyte TNF-alpha induction by bacterial lipopeptides, lipopolysaccharide, and imiquimod, but preserves the response to R-848. J Immunol 173(7):4627–4634

Lindsey NP, Schroeder BA, Miller ER, Braun MM, Hinckley AF, Marano N, Slade BA, Barnett ED, Brunette GW, Horan K, Staples JE, Kozarsky PE, Hayes EB (2008) Adverse event reports following yellow fever vaccination. Vaccine 26(48):6077–6082. doi:10.1016/j.vaccine.2008.09.009

Lisse IM, Aaby P, Whittle H, Jensen H, Engelmann M, Christensen LB (1997) T-lymphocyte subsets in West African children: impact of age, sex, and season. J Pediatr 130(1):77–85

Liu CA, Wang CL, Chuang H, Ou CY, Hsu TY, Yang KD (2003) Prediction of elevated cord blood IgE levels by maternal IgE levels, and the neonate's gender and gestational age. Chang Gung Med J 26(8):561–569

Loken MO, Jeansson S, Jenum PA, Eskild A (2010) Serum level of immunoglobulin E during pregnancy - does offspring sex matter? Paediatr Perinat Epidemiol 24(1):75–78. doi:10.1111/j.1365-3016.2009.01092.x

Lund N, Andersen A, Monteiro I, Aaby P, Benn CS (2012) No effect of oral polio vaccine administered at birth on mortality and immune response to BCG. A natural experiment. Vaccine 30(47):6694–6699. doi:10.1016/j.vaccine.2012.08.055

Lyamuya EF, Matee MI, Aaby P, Scheutz F (1999) Serum levels of measles IgG antibody activity in children under 5 years in Dar-es-Salaam, Tanzania. Ann Trop Paediatr 19(2):175–183

Maat M, Buysse CM, Emonts M, Spanjaard L, Joosten KF, de Groot R, Hazelzet JA (2007) Improved survival of children with sepsis and purpura: effects of age, gender, and era. Critical Care (London, England) 11(5):R112. doi:10.1186/cc6161

Maini MK, Gilson RJ, Chavda N, Gill S, Fakoya A, Ross EJ, Phillips AN, Weller IV (1996) Reference ranges and sources of variability of CD4 counts in HIV-seronegative women and men. Genitourin Med 72(1):27–31

Marguerite M, Gallissot MC, Diagne M, Moreau C, Diakkhate MM, Roberts M, Remoue F, Thiam A, Decam C, Rogerie F, Cottrez F, Neyrinck JL, Butterworth AE, Sturrock RF, Piau JP, Daff B, Niang M, Wolowczuk I, Riveau G, Auriault C, Capron A (1999) Cellular immune responses of a Senegalese community recently exposed to Schistosoma mansoni: correlations of infection level with age and inflammatory cytokine production by soluble egg antigen-specific cells. Trop Med Int Health 4(8):530–543

Mark A, Carlsson RM, Granstrom M (1999) Subcutaneous versus intramuscular injection for booster DT vaccination of adolescents. Vaccine 17(15–16):2067–2072

Markowitz LE, Albrecht P, Rhodes P, Demonteverde R, Swint E, Maes EF, Powell C, Patriarca PA (1996) Changing levels of measles antibody titers in women and children in the United States: impact on response to vaccination. Kaiser Permanente Measles Vaccine Trial Team. Pediatrics 97(1):53–58

Marriott I, Bost KL, Huet-Hudson YM (2006) Sexual dimorphism in expression of receptors for bacterial lipopolysaccharides in murine macrophages: a possible mechanism for gender-based differences in endotoxic shock susceptibility. J Reprod Immunol 71(1):12–27. doi:10.1016/j.jri.2006.01.004

Martinez-Mir I, Garcia-Lopez M, Palop V, Ferrer JM, Rubio E, Morales-Olivas FJ (1999) A prospective study of adverse drug reactions in hospitalized children. Br J Clin Pharmacol 47(6):681–688

Martins C, Bale C, Garly ML, Rodrigues A, Lisse IM, Andersen A, Eriksson M, Benn CS, Whittle H, Aaby P (2009) Girls may have lower levels of maternal measles antibodies and higher risk of subclinical measles infection before the age of measles vaccination. Vaccine 27(38):5220–5225

Martins C, Garly ML, Bale C, Rodrigues A, Benn CS, Whittle H, Aaby P (2013) Measles antibody levels after vaccination with Edmonston-Zagreb and Schwarz measles vaccine at 9 months or at 9 and 18 months of age: a serological study within a randomised trial of different measles vaccines. Vaccine 31(48):5766–5771. doi:10.1016/j.vaccine.2013.08.044

McBrien J, Murphy J, Gill D, Cronin M, O'Donovan C, Cafferkey MT (2003) Measles outbreak in Dublin, 2000. Pediatr Infect Dis J 22(7):580–584. doi:10.1097/01.inf.0000073059.57867.36

McMahon BJ, Williams J, Bulkow L, Snowball M, Wainwright R, Kennedy M, Krause D (1995) Immunogenicity of an inactivated hepatitis A vaccine in Alaska Native children and Native and non-Native adults. J Infect Dis 171(3):676–679

Miller EK, Bugna J, Libster R, Shepherd BE, Scalzo PM, Acosta PL, Hijano D, Reynoso N, Batalle JP, Coviello S, Klein MI, Bauer G, Benitez A, Kleeberger SR, Polack FP (2012) Human rhinoviruses in severe respiratory disease in very low birth weight infants. Pediatrics 129(1):e60–e67. doi:10.1542/peds. 2011-0583

Mohammed I, Damisah MM (1982) The immunological response to polyvalent meningococcal vaccine in Bauchi State, Nigeria. Trans R Soc Trop Med Hyg 76(3):351–353

Molbak K, Jensen H, Ingholt L, Aaby P (1997) Risk factors for diarrheal disease incidence in early childhood: a community cohort study from Guinea-Bissau. Am J Epidemiol 146(3): 273–282

Moore SE, Goldblatt D, Bates CJ, Prentice AM (2003) Impact of nutritional status on antibody responses to different vaccines in undernourished Gambian children. Acta Paediatrica (Oslo, Norway : 1992) 92(2):170–176

Moore SE, Collinson AC, Fulford AJ, Jalil F, Siegrist CA, Goldblatt D, Hanson LA, Prentice AM (2006) Effect of month of vaccine administration on antibody responses in The Gambia and Pakistan. Trop Med Int Health 11(10):1529–1541. doi:10.1111/j.1365-3156.2006.01700.x

Moore SE, Fulford AJ, Wagatsuma Y, Persson LA, Arifeen SE, Prentice AM (2014) Thymus development and infant and child mortality in rural Bangladesh. Int J Epidemiol 43(1):216–223. doi:10.1093/ije/dyt232

Morris SK, Awasthi S, Kumar R, Shet A, Khera A, Nakhaee F, Ram U, Brandao JR, Jha P (2013) Measles mortality in high and low burden districts of India: estimates from a nationally representative study of over 12,000 child deaths. Vaccine 31(41):4655–4661. doi:10.1016/j.vaccine.2013.07.012

Muller-Werdan U, Wilhelm J, Hettwer S, Nuding S, Ebelt H, Werdan K (2009) Specific aspects in septic patients: initial phase in the emergency department, age, sex and post-ICU-care. Internist 50(7):828. doi:10.1007/s00108-008-2288-4, 830–822, 834–826

Mullick S, Rusia U, Sikka M, Faridi MA (2006) Impact of iron deficiency anaemia on T lymphocytes & their subsets in children. Ind J Med Res 124(6):647–654

Nagayama Y, Tsubaki T, Nakayama S, Sawada K, Taguchi K, Tateno N, Toba T (2006) Gender analysis in acute bronchiolitis due to respiratory syncytial virus. Pediatr Allerg Immunol 17(1):29–36. doi:10.1111/j.1399-3038.2005.00339.x

Nath DC, Goswami G (1997) Determinants of breast-feeding patterns in an urban society of India. Hum Biol 69(4):557–573

Ndhlovu Z, Ryon JJ, Griffin DE, Monze M, Kasolo F, Moss WJ (2004) CD4+ and CD8+ T-lymphocyte subsets in Zambian children. J Trop Pediatr 50(2):94–97

Neubauer V, Griesmaier E, Ralser E, Kiechl-Kohlendorfer U (2012) The effect of sex on outcome of preterm infants - a population-based survey. Acta Paediatrica (Oslo, Norway: 1992) 101(9): 906–911. doi:10.1111/j.1651-2227.2012.02709.x

Neyrolles O, Quintana-Murci L (2009) Sexual inequality in tuberculosis. PLoS Med 6(12): e1000199. doi:10.1371/journal.pmed.1000199

Ngom PT, Collinson AC, Pido-Lopez J, Henson SM, Prentice AM, Aspinall R (2004) Improved thymic function in exclusively breastfed infants is associated with higher interleukin 7 concentrations in their mothers' breast milk. Am J Clin Nutr 80(3):722–728

Nguyen M, Leuridan E, Zhang T, De Wit D, Willems F, Van Damme P, Goldman M, Goriely S (2010) Acquisition of adult-like TLR4 and TLR9 responses during the first year of life. PLoS ONE 5(4):e10407. doi:10.1371/journal.pone.0010407

Nicoara C, Zach K, Trachsel D, Germann D, Matter L (1999) Decay of passively acquired maternal antibodies against measles, mumps, and rubella viruses. Clin Diagn Lab Immunol 6(6):868–871

Njie-Jobe J, Nyamweya S, Miles DJ, van der Sande M, Zaman S, Touray E, Hossin S, Adetifa J, Palmero M, Burl S, Jeffries D, Rowland-Jones S, Flanagan K, Jaye A, Whittle H (2012) Immunological impact of an additional early measles vaccine in Gambian children: responses to a boost at 3 years. Vaccine 30(15):2543–2550. doi:10.1016/j.vaccine.2012.01.083

Nzolo D, Ntetani Aloni M, Mpiempie Ngamasata T, Mvete Luemba B, Bazundama Marfeza S, Bothale Ekila M, Ndosimao Nsibu C, Lutete Tona N (2013) Adverse events following immunization with oral poliovirus in Kinshasa, Democratic Republic of Congo: preliminary results. Pathog Glob Health 107(7):381–384

Okan F, Karagoz S, Nuhoglu A (2006) Bacillus Calmette-Guerin vaccination in preterm infants. Int J Tuberc Lung Dis 10(12):1337–1341

Omoregie R, Egbe C, Ogefere H, Igbarumah I, Omijie R (2009) Effects of gender and seasonal variation on the prevalence of bacterial septicemia among young children in Benin City, Nigeria. Libyan J Med 4(3):107–109. doi:10.4176/090206

Osrin D, Vaidya A, Shrestha Y, Baniya RB, Manandhar DS, Adhikari RK, Filteau S, Tomkins A, Costello AM (2005) Effects of antenatal multiple micronutrient supplementation on birthweight and gestational duration in Nepal: double-blind, randomised controlled trial. Lancet 365(9463):955–962. doi:10.1016/s0140-6736(05)71084-9

Palmeira P, Quinello C, Silveira-Lessa AL, Zago CA, Carneiro-Sampaio M (2012) IgG placental transfer in healthy and pathological pregnancies. Clin Dev Immunol 2012:985646. doi:10.1155/2012/985646

Pathak S, Rege M, Gogtay NJ, Aigal U, Sharma SK, Valecha N, Bhanot G, Kshirsagar NA, Sharma S (2012) Age-dependent sex bias in clinical malarial disease in hypoendemic regions. PLoS ONE 7(4):e35592. doi:10.1371/journal.pone.0035592

Penny ME (2013) Zinc supplementation in public health. Annal Nutr Metabol 62(Suppl 1):31–42. doi:10.1159/000348263

Perch M, Sodemann M, Jakobsen MS, Valentiner-Branth P, Steinsland H, Fischer TK, Lopes DD, Aaby P, Molbak K (2001) Seven years' experience with Cryptosporidium parvum in Guinea-Bissau, West Africa. Ann Trop Paediatr 21(4):313–318

Quach C, Piche-Walker L, Platt R, Moore D (2003) Risk factors associated with severe influenza infections in childhood: implication for vaccine strategy. Pediatrics 112(3 Pt 1):e197–e201

Rahmathullah L, Tielsch JM, Thulasiraj RD, Katz J, Coles C, Devi S, John R, Prakash K, Sadanand AV, Edwin N, Kamaraj C (2003) Impact of supplementing newborn infants with vitamin A on early infant mortality: community based randomised trial in southern India. BMJ (Clin Res Ed) 327(7409):254. doi:10.1136/bmj.327.7409.254

Rajan TV, Nelson FK, Shultz LD, Shultz KL, Beamer WG, Yates J, Greiner DL (1994) Influence of gonadal steroids on susceptibility to Brugia malayi in scid mice. Acta Trop 56(4):307–314

Reif DM, McKinney BA, Motsinger AA, Chanock SJ, Edwards KM, Rock MT, Moore JH, Crowe JE (2008) Genetic basis for adverse events after smallpox vaccination. J Infect Dis 198(1):16–22. doi:10.1086/588670

Ribeiro-Vaz I, Marques J, Demoly P, Polonia J, Gomes ER (2013) Drug-induced anaphylaxis: a decade review of reporting to the Portuguese Pharmacovigilance Authority. Eur J Clin Pharmacol 69(3):673–681. doi:10.1007/s00228-012-1376-5

Rodrigues A, Fischer TK, Valentiner-Branth P, Nielsen J, Steinsland H, Perch M, Garly ML, Molbak K, Aaby P (2006) Community cohort study of rotavirus and other enteropathogens: are routine vaccinations associated with sex-differential incidence rates? Vaccine 24(22): 4737–4746. doi:10.1016/j.vaccine.2006.03.033

Roth A, Sodemann M, Jensen H, Poulsen A, Gustafson P, Gomes J, Djana Q, Jakobsen M, Garly ML, Rodrigues A, Aaby P (2005) Vaccination technique, PPD reaction and BCG scarring in a cohort of children born in Guinea-Bissau 2000–2002. Vaccine 23(30):3991–3998. doi:10.1016/j.vaccine.2004.10.022

Roth A, Garly ML, Jensen H, Nielsen J, Aaby P (2006) Bacillus Calmette-Guerin vaccination and infant mortality. Expert Rev Vaccines 5(2):277–293. doi:10.1586/14760584.5.2.277

Ruz M, Castillo-Duran C, Lara X, Codoceo J, Rebolledo A, Atalah E (1997) A 14-mo zinc-supplementation trial in apparently healthy Chilean preschool children. Am J Clin Nutr 66(6): 1406–1413

Ryon JJ, Moss WJ, Monze M, Griffin DE (2002) Functional and phenotypic changes in circulating lymphocytes from hospitalized Zambian children with measles. Clin Diagn Lab Immunol 9(5): 994–1003

Sartono E, Lisse IM, Terveer EM, van de Sande PJ, Whittle H, Fisker AB, Roth A, Aaby P, Yazdanbakhsh M, Benn CS (2010) Oral polio vaccine influences the immune response to

BCG vaccination. A natural experiment. PLoS ONE 5(5):e10328. doi:10.1371/journal.pone.0010328

Savy M, Edmond K, Fine PE, Hall A, Hennig BJ, Moore SE, Mulholland K, Schaible U, Prentice AM (2009) Landscape analysis of interactions between nutrition and vaccine responses in children. J Nutr 139(11):2154S–2218S. doi:10.3945/jn.109.105312

Sawyer CC (2012) Child mortality estimation: estimating sex differences in childhood mortality since the 1970s. PLoS Med 9(8):e1001287. doi:10.1371/journal.pmed.1001287

Schnorr JJ, Cutts FT, Wheeler JG, Akramuzzaman SM, Alam MS, Azim T, Schneider-Schaulies S, ter-Meulen V (2001) Immune modulation after measles vaccination of 6–9 months old Bangladeshi infants. Vaccine 19(11–12):1503–1510

Semba RD, Munasir Z, Beeler J, Akib A, Muhilal AS, Sommer A (1995) Reduced seroconversion to measles in infants given vitamin A with measles vaccination. Lancet 345(8961):1330–1332

Seng R, Samb B, Simondon F, Cisse B, Soumare M, Jensen H, Bennett J, Whittle H, Aaby P (1999) Increased long term mortality associated with rash after early measles vaccination in rural Senegal. Pediatr Infect Dis J 18(1):48–52

Seppala E, Viskari H, Hoppu S, Honkanen H, Huhtala H, Simell O, Ilonen J, Knip M, Hyoty H (2011) Viral interference induced by live attenuated virus vaccine (OPV) can prevent otitis media. Vaccine 29(47):8615–8618. doi:10.1016/j.vaccine.2011.09.015

Shaheen SO, Aaby P, Hall AJ, Barker DJ, Heyes CB, Shiell AW, Goudiaby A (1996) Cell mediated immunity after measles in Guinea-Bissau: historical cohort study. BMJ (Clin Res Ed) 313(7063):969–974

Shankar AH, Prasad AS (1998) Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 68(2 Suppl):447S–463S

Shann F (2010) The non-specific effects of vaccines. Arch Dis Child 95(9):662–667. doi:10.1136/adc.2009.157537

Shiddo SA, Aden Mohamed A, Akuffo HO, Mohamud KA, Herzi AA, Herzi Mohamed H, Huldt G, Nilsson LA, Ouchterlony O, Thorstensson R (1995) Visceral leishmaniasis in Somalia: prevalence of markers of infection and disease manifestations in a village in an endemic area. Trans R Soc Trop Med Hyg 89(4):361–365

Shohat T, Green MS, Nakar O, Ballin A, Duvdevani P, Cohen A, Shohat M (2000) Gender differences in the reactogenicity of measles-mumps-rubella vaccine. Isr Med Assoc J 2(3):192–195

Siddiqui FQ, Ahmad MM, Kakar F, Akhtar S, Dil AS (2001) The role of vitamin A in enhancing humoral immunity produced by antirabies vaccine. East Mediterr Health J 7(4–5):799–804

Siegrist CA (2003) Mechanisms by which maternal antibodies influence infant vaccine responses: review of hypotheses and definition of main determinants. Vaccine 21(24):3406–3412

Siegrist CA, Barrios C, Martinez X, Brandt C, Berney M, Cordova M, Kovarik J, Lambert PH (1998) Influence of maternal antibodies on vaccine responses: inhibition of antibody but not T cell responses allows successful early prime-boost strategies in mice. Eur J Immunol 28(12):4138–4148

Sinha A, Madden J, Ross-Degnan D, Soumerai S, Platt R (2003) Reduced risk of neonatal respiratory infections among breastfed girls but not boys. Pediatrics 112(4):e303

Siziya S, Muula AS, Rudatsikira E (2013) Correlates of diarrhoea among children below the age of 5 years in Sudan. Afr Health Sci 13(2):376–383. doi:10.4314/ahs.v13i2.26

Soofi S, Cousens S, Iqbal SP, Akhund T, Khan J, Ahmed I, Zaidi AK, Bhutta ZA (2013) Effect of provision of daily zinc and iron with several micronutrients on growth and morbidity among young children in Pakistan: a cluster-randomised trial. Lancet 382(9886):29–40. doi:10.1016/s0140-6736(13)60437-7

Star K, Noren GN, Nordin K, Edwards IR (2011) Suspected adverse drug reactions reported for children worldwide: an exploratory study using VigiBase. Drug Safety 34(5):415–428. doi:10.2165/11587540-000000000-00000

Stark MJ, Clifton VL, Wright IM (2008) Sex-specific differences in peripheral microvascular blood flow in preterm infants. Pediatr Res 63(4):415–419. doi:10.1203/01.pdr.0000304937.38669.63

Stensballe LG, Nante E, Jensen IP, Kofoed PE, Poulsen A, Jensen H, Newport M, Marchant A, Aaby P (2005) Acute lower respiratory tract infections and respiratory syncytial virus in infants in Guinea-Bissau: a beneficial effect of BCG vaccination for girls community based case–control study. Vaccine 23(10):1251–1257. doi:10.1016/j.vaccine.2004.09.006

Stern DA, Hicks MJ, Martinez FD, Holberg CJ, Wright AL, Pinnas J, Halonen M, Taussig LM (1992) Lymphocyte subpopulation number and function in infancy. Dev Immunol 2(3):175–179

Stevenson DK, Verter J, Fanaroff AA, Oh W, Ehrenkranz RA, Shankaran S, Donovan EF, Wright LL, Lemons JA, Tyson JE, Korones SB, Bauer CR, Stoll BJ, Papile LA (2000) Sex differences in outcomes of very low birthweight infants: the newborn male disadvantage. Arch Dis Child Fetal Neonatal Ed 83(3):F182–F185

Strachan NJ, Watson RO, Novik V, Hofreuter D, Ogden ID, Galan JE (2008) Sexual dimorphism in campylobacteriosis. Epidemiol Infect 136(11):1492–1495. doi:10.1017/s0950268807009934

Suchdev PS, Ruth LJ, Woodruff BA, Mbakaya C, Mandava U, Flores-Ayala R, Jefferds ME, Quick R (2012) Selling Sprinkles micronutrient powder reduces anemia, iron deficiency, and vitamin A deficiency in young children in Western Kenya: a cluster-randomized controlled trial. Am J Clin Nutr 95(5):1223–1230. doi:10.3945/ajcn.111.030072

Thibault H, Galan P, Selz F, Preziosi P, Olivier C, Badoual J, Hercberg S (1993) The immune response in iron-deficient young children: effect of iron supplementation on cell-mediated immunity. Eur J Pediatr 152(2):120–124

Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, Slagboom PE, Heijmans BT (2009) DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Hum Mol Genet 18(21):4046–4053. doi:10.1093/hmg/ddp353

Tollerud DJ, Clark JW, Brown LM, Neuland CY, Pankiw-Trost LK, Blattner WA, Hoover RN (1989) The influence of age, race, and gender on peripheral blood mononuclear-cell subsets in healthy nonsmokers. J Clin Immunol 9(3):214–222

Ulizzi L, Zonta LA (2002) Sex differential patterns in perinatal deaths in Italy. Hum Biol 74(6):879–888

Ulrey CL, Liu L, Andrews LG, Tollefsbol TO (2005) The impact of metabolism on DNA methylation. Hum Mol Genet 14(Spec No 1):R139–R147. doi:10.1093/hmg/ddi100

Umlauf BJ, Haralambieva IH, Ovsyannikova IG, Kennedy RB, Pankratz VS, Jacobson RM, Poland GA (2012) Associations between demographic variables and multiple measles-specific innate and cell-mediated immune responses after measles vaccination. Viral Immunol 25(1):29–36. doi:10.1089/vim.2011.0051

UN (2013) www.un.org/millenniumgoals/childhealth.shtml.

Uppal SS, Verma S, Dhot PS (2003) Normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effects of sex, age, ethnicity, and smoking. Cytometry B Clin Cytom 52(1):32–36. doi:10.1002/cyto.b.10011

Valentiner-Branth P, Steinsland H, Fischer TK, Perch M, Scheutz F, Dias F, Aaby P, Molbak K, Sommerfelt H (2003) Cohort study of Guinean children: incidence, pathogenicity, conferred protection, and attributable risk for enteropathogens during the first 2 years of life. J Clin Microbiol 41(9):4238–4245. doi:10.1128/jcm. 41.9.4238-4245.2003

Vatten LJ, Skjaerven R (2004) Offspring sex and pregnancy outcome by length of gestation. Early Hum Dev 76(1):47–54

Virtanen M, Peltola H, Paunio M, Heinonen OP (2000) Day-to-day reactogenicity and the healthy vaccinee effect of measles-mumps-rubella vaccination. Pediatrics 106(5):E62

Watson RS, Carcillo JA, Linde-Zwirble WT, Clermont G, Lidicker J, Angus DC (2003) The epidemiology of severe sepsis in children in the United States. Am J Respir Crit Care Med 167(5):695–701. doi:10.1164/rccm.200207-682OC

Weise V-J (1979) Imported cases of malaria into the Federal Republic of Germany and West Berlin with particular emphasis upon the last 5 years, 1973–77 [translated from German]. Bundesgesundheitsblatt 22:1–7

Were T, Davenport GC, Hittner JB, Ouma C, Vulule JM, Ong'echa JM, Perkins DJ (2011) Bacteremia in Kenyan children presenting with malaria. J Clin Microbiol 49(2):671–676. doi:10.1128/jcm. 01864-10

WHO (2011) Guideline: intermittent iron supplementation in preschool and school-age children. World Health Organization 2011, Geneva

WHO (2012) World Health Statistics 2012. WHO Global Health Observatory

Wieringa FT, Berger J, Dijkhuizen MA, Hidayat A, Ninh NX, Utomo B, Wasantwisut E, Winichagoon P (2007) Sex differences in prevalence of anaemia and iron deficiency in infancy in a large multi-country trial in South-East Asia. Br J Nutr 98(5):1070–1076. doi:10.1017/s0007114507756945

Winter JS, Faiman C, Hobson WC, Prasad AV, Reyes FI (1975) Pituitary-gonadal relations in infancy. I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. J Clin Endocrinol Metabol 40(4):545–551. doi:10.1210/jcem-40-4-545

Wintergerst ES, Maggini S, Hornig DH (2007) Contribution of selected vitamins and trace elements to immune function. Annal Nutr Metabol 51(4):301–323. doi:10.1159/000107673

Wong WS, Lo AW, Siu LP, Leung JN, Tu SP, Tai SW, Lam SC, Wong KF (2013) Reference ranges for lymphocyte subsets among healthy Hong Kong Chinese adults by single-platform flow cytometry. Clin Vaccine Immunol 20(4):602–606. doi:10.1128/cvi. 00476-12

Yakymenko D, Benn CS, Martins C, Diness BR, Fisker AB, Rodrigues A, Aaby P (2011) The impact of different doses of vitamin A supplementation on male and female mortality. A randomised trial from Guinea-Bissau. BMC Pediatr 11:77. doi:10.1186/1471-2431-11-77

Yan SR, Qing G, Byers DM, Stadnyk AW, Al-Hertani W, Bortolussi R (2004) Role of MyD88 in diminished tumor necrosis factor alpha production by newborn mononuclear cells in response to lipopolysaccharide. Infect Immun 72(3):1223–1229

Chapter 11
Reproductive Tract Infections in Women

Rebecca M. Brotman and Khalil G. Ghanem

Abstract Reproductive tract infections (RTIs) are of significant public health concern. The bulk of their long-term morbidity disproportionately affects women. Consequently, research and public health efforts have focused on trying to minimize the burden of these infections in women. In this chapter, we focus on two agents that cause RTIs: *Neisseria gonorrhoeae*, a curable bacterial infection, and herpes simplex virus (HSV), a viral pathogen for which no cure exists. We have chosen these pathogens to highlight intriguing differences in epidemiology, clinical manifestations, and immunological responses noted between men and women. We begin by summarizing what we know about these infections in women and any existing differences between the sexes. Potential mechanisms that may explain the sex-based differences observed for these and other RTIs are evaluated. Such mechanisms consist of both behavioral (gender) and biological (sex) factors. Consideration of the role of mucosal immune responses, sex hormones, and the vaginal microbiome in mediating sex-based differences are suggested.

11.1 Introduction

Reproductive tract infections (RTIs) are of significant public health concern. The bulk of their long-term morbidity disproportionately occurs in women. Two major goals of targeting these infections are to decrease the reproductive sequelae in women and to decrease the overall risk of HIV acquisition and transmission. Several infections, including *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis*

R.M. Brotman
Department of Epidemiology and Public Health, Institute for Genome Sciences, University of Maryland School of Medicine, BioPark Building II, 801 West Baltimore Street, Room Number 627, Baltimore, MD 21201, USA
e-mail: rbrotman@som.umaryland.edu

K.G. Ghanem (✉)
Division of Infectious Diseases, Johns Hopkins University School of Medicine, JHUBMC, 5200 Eastern Ave, MFL Center Tower #378, Baltimore, MD 21224, USA
e-mail: kghanem@jhmi.edu

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_11

(CT), *Mycoplasma genitalium*, and bacterial vaginosis (BV), have been associated with an increased risk of pelvic inflammatory disease (PID), an infection of the upper reproductive tract of women (i.e., the uterus, fallopian tubes, and ovaries), which leads to inflammation and scarring (Trent 2013). The sequelae of PID include infertility, tubo-ovarian abscesses, and chronic debilitating pelvic pain. The same RTIs are also associated with increased risk of HIV acquisition and transmission (Cohen et al. 2012; Røttingen et al. 2001). Consequently, research and public health efforts have focused on trying to minimize the burden of these infections in women.

Beyond their impact on reproductive outcomes, some striking sex-based differences in epidemiology, clinical manifestations, and immunological responses to infection exist. For example, disseminated gonococcal infection (DGI), a complication of NG infection associated with high mortality, is four times more common in women than men (O'Brien et al. 1983). Herpes simplex virus (HSV), for which no screening recommendations exist and which has been associated with a threefold increased risk of HIV transmission, is twice as prevalent in women as in men (Xu et al. 2006; Looker et al. 2008).

In this chapter, we will focus on several agents that cause RTIs: NG, a curable bacterial infection, and HSV, a viral pathogen for which no cure exists. We have chosen these pathogens to highlight intriguing differences in epidemiology, clinical manifestations, and immunological responses noted between men and women. We will begin by summarizing what we know about these infections in women and any existing differences by sex. This will help set the stage to review potential mechanisms that may help to explain the sex-based differences observed for these and other RTIs. Such mechanisms consist of both behavioral and biological factors, including contributions from the vaginal microbiota, sex hormones, and local mucosal immune responses.

11.2 Epidemiology, Clinical Manifestations, and Sex-Based Differences for Select Bacterial and Viral Reproductive Tract Infections

11.2.1 Neisseria gonorrhoeae

Despite significant declines in new cases of gonorrhea in the past 30 years, it remains the second most commonly reported infectious disease in the United States (Division of Std Prevention 2013). In 2012, 334,826 cases were reported. These numbers most likely underestimate the true prevalence of gonorrhea. It is believed that approximately twice as many new infections occur each year as are reported. Estimates of disease burden are even less reliable in many developing countries. Available data suggest, however, that gonorrhea remains relatively common in the developing world. According to the World Health Organization, the global

estimated incidence of gonorrhea is 106 million new infections annually (World Health Organization 2009).

Young age is a risk factor for gonorrhea, as it is for most STDs; half of all new sexually transmitted infections occur in young people 15–24 years of age (Division of Std Prevention 2013). According to the 2012 CDC surveillance data derived from clinicians and public health and commercial laboratories throughout the United States, the highest rate of gonococcal infections occurred among individuals 20–24 years of age. Young women seem to be at particularly high risk for gonorrhea. In 2012, women aged 15–19 and 20–24 years had the highest reported rates of gonococcal infection: 521.2 and 578.5 cases per 100,000, respectively. This is in contrast to young men whose rates were 239 and 462.8 per 100,000, respectively. These data are biased toward increased testing in symptomatic men and women. Annual screening for gonorrhea is recommended for asymptomatic high-risk men and women. Factors that increase risk include young age, a previous history of gonorrhea or other sexually transmitted diseases, having new or multiple sex partners, using condoms inconsistently, working in the commercial sex industry, using drugs, or living in communities with a high prevalence of disease. In the United States, fewer than 20 % of high-risk individuals who should be screened for gonorrhea are tested.

Infections caused by NG are typically limited to superficial mucosal surfaces lined by columnar or cuboidal, nonkeratinized epithelial cells. Infection of these mucosal surfaces is usually accompanied by a marked inflammatory response. NG can infect several anatomic sites, including the cervix, urethra, rectum, oropharynx, and conjunctiva. An individual with gonorrhea may have no symptoms at all, may have localized symptomatic disease, may have localized complicated disease, or may be very ill with DGI and bacteremia. Of men and women who report sexual exposure to partners with gonorrhea, urethral infection in men is asymptomatic up to 60 % of the time and cervical infection in women is asymptomatic up to 80 % of the time. Pharyngeal and rectal infections are typically asymptomatic in up to 90 % of cases (Workowski 2013).

The most common form of uncomplicated gonorrhea in women is cervicitis. The vagina is usually not infected because it is lined by a squamous epithelium. The urethra is colonized in 70–90 % of infected women with NG infection. Symptoms, should they occur, appear within 10 days of infection, although the incubation period can vary. The symptoms associated with endocervical infection may include vaginal discharge, genital itching, intermenstrual bleeding, unusually heavy menstrual bleeding, or painful urination. An infected woman may have all, none, or any combination of these symptoms; and symptoms may range from mild to severe. Physical examination of an infected woman may be normal, but may reveal a purulent discharge from the cervix, redness and swelling of the cervix, and easily induced cervical bleeding. These signs and symptoms are not specific for gonorrhea.

PID is one of the most serious complications of NG infection in women, affecting approximately 10–20 % of infected women. Gonococcal PID results from the spread of the organism into the upper genital tract. Symptoms of PID

can include fever, unilateral or bilateral lower abdominal pain, pain associated with sexual intercourse, abnormal menses or intermenstrual bleeding, or other complaints associated with an intra-abdominal infection. These symptoms may be severe or mild. Pregnant women with genital gonorrhea are at significant risk for spontaneous abortion, premature rupture of membranes, premature delivery, and acute chorioamnionitis, as well as for transmitting gonorrhea to their newborns during delivery (Liu et al. 2013; Johnson et al. 2011). Neonates infected with gonorrhea can suffer eye and pharyngeal involvement, as well as other complications.

DGI occurs in approximately 1–2 % of individuals with untreated gonorrhea as a result of the spread of the organism from typically asymptomatic mucosal infections of the pharynx, cervix, urethra, or rectum into the bloodstream (O'Brien et al. 1983; Rice 2005; Handsfield 1975). Most DGI studies were conducted in large urban areas in the United States and Western Europe and involved a case series limited to a single hospital or clinic. There are limited population-based data. Bacteremia probably begins 7–30 days after initial infection. DGI is more common in women than in men. The sex ratio was 4:1 in most European and US studies conducted in the second half of the twentieth century. Recently, a 3-year retrospective analysis of 21 DGI cases collected through two networks of microbiologists and infectious disease specialists in France between 2009 and 2011 reported that 60 % of patients with DGI were men (Belkacem et al. 2013). This finding is in stark contrast to the majority of published data that suggest that most infections occur in women. Indeed, up to over 90 % of patients included in these series were women. This is attributed to several factors: the greater likelihood that women with mucosal infections are asymptomatic and thus do not present for treatment, menstrual blood that may act as a growth promoter given the observation that women are more likely to develop the first manifestations of DGI a few days after the onset of menstruation, sex hormones that may suppress immune responses around the time of menstruation (Britigan et al. 1985), and the greater prevalence of certain immunodeficiencies such as terminal complement deficiencies among women that may increase their risk for DGI (Petersen et al. 1979).

Up to 40 % of women in the DGI series were pregnant. Among pregnant women, the risk of DGI increases with the progression of pregnancy (Phupong et al. 2005). This has been hypothesized to be related to increased immunosuppression later in the course of pregnancy, increased mucosal vascularity, the influence of sex hormones, and selection of virulent gonococcal strains.

DGI is often referred to as an arthritis/dermatitis syndrome, given the usual findings of an asymmetric polyarthritis and associated rash. The joint manifestations in DGI may begin with painful joints, which progress to frank arthritis later in the course of disease. Approximately 30–40 % of patients with DGI will present with overt arthritis, usually involving the wrist, metatarsophalangeal, ankle, or knee joints, although any joint may be involved. Rarely, seeding of the meninges or the heart valves can further complicate DGI, resulting in potentially life-threatening gonococcal meningitis or endocarditis. DGI can also occur in newborns of infected mothers, when exposed to secretions during vaginal delivery. The illness in

neonates can be similar to that in adults, with associated meningitis and arthritis (Wise et al. 1994; Rompalo et al. 1987).

11.2.2 Herpes Simplex Viruses

Genital herpes is one of the most prevalent sexually transmitted infections and is the leading cause of genital ulcer diseases worldwide (Xu et al. 2006; Looker et al. 2008). HSV-1 and HSV-2 both cause genital herpes. HSV-1 also causes orolabial infections, so the detection of antibodies to HSV-1 in serum does not distinguish between genital and orolabial infections. Consequently, population rates of genital HSV-1 infections are not well established. Up to 70 % of HSV infections are asymptomatic. Globally, the World Health Organization estimates that 24 million people are newly infected annually with HSV-2 and that 536 million 15–49-year-olds are living with it. Herpes is not a reportable infection in the United States. The estimated annual incidence of HSV-2 infection in the United States is 776,000 new infections and the total number of HSV-2 infected persons is estimated to be 24 million. Recent studies suggest that the majority of incident genital herpes infections among young people are due to HSV-1 (Looker and Garnett 2005). Among adult women enrolled in a vaccine study who were HSV-1 uninfected, 84 % of newly acquired HSV-1 infections were genital (Bernstein et al. 2013). Acquisition of HSV-1 infections in persons with prior HSV-2 infections is rare. Previous HSV-1 infection does not reduce the rate of HSV-2 infection, but it does increase the likelihood of asymptomatic HSV-2 seroconversion threefold. Viral shedding occurs even in the absence of lesions. In the first year following HSV-2 infection, viral shedding occurs on about 30 % of days (Tronstein et al. 2011). Viral shedding tends to decrease with time. HSV-2 infection is estimated to triple the risk of HIV transmission (Freeman et al. 2006).

The best US population estimates are derived from the National Health and Nutritional Examination Survey (NHANES) serosurvey (Xu et al. 2006). The overall age-adjusted HSV-2 seroprevalence was 17.0 % in NHANES 1999–2004 and 21.0 % in 1988–1994, a relative decrease of 19.0 % between the two surveys. Seroprevalence of HSV-1 decreased from 62.0 % in 1988–1994 to 57.7 % in 1999–2004, a relative decrease of 6.9 % between the two surveys. Differences by sex are striking and increased between NHANES 1988–1994 and NHANES 1999–2004. Among men, 17 % and 11.2 % were infected in NHANES 1988–1994 and NHANES 1999–2004, respectively. Among women, 25.2 % and 22.8 % were infected, respectively. Similar sex differences have been demonstrated elsewhere around the world. The reasons for the higher prevalence among women are unclear. One possible reason is the anatomical differences between women and men rendering women more susceptible to infection. Although there are no studies that have tested this hypothesis, studies assessing other sexually transmitted infections such as gonorrhea, chlamydia, and HIV have found that transmission from men to women is more efficient than transmission from women to men. In one small study assessing HSV-2 serodiscordant monogamous couples (i.e., where one member is

seropositive and the other is not), seronegative women were much more likely to acquire HSV-2 infection from their infected male partner compared to men (Bryson et al. 1993). Alternatively, differences in the pattern of mixing between the genders may expose women to a higher prevalence of infection at younger ages. Differences in the distribution of sexual risk behaviors between men and women may also contribute to this difference.

There is no cure for genital herpes. Antiviral drugs are available that decrease the severity and duration of symptoms, decrease viral shedding, and help decrease the risk of transmission from an infected partner to an uninfected one (Schiffer et al. 2011). The use of antiviral drugs and condoms helps to decrease the risk of heterosexual transmission by about 55 % (Corey et al. 2004).

Several vaccine candidates have been tested with limited success. Two phase III trials of an adjuvanted recombinant glycoprotein D2 vaccine appeared to generate protection against genital herpes disease and demonstrated a trend toward protection from infection with HSV-2, but only in HSV-1 seronegative women (Stanberry et al. 2002). The vaccine provided no protection in men. Given these findings in women, another trial was conducted to evaluate the efficacy of this vaccine among 8323 HSV-1 and HSV-2 uninfected women (Belshe et al. 2012). Overall the vaccine was not found to be protective against HSV-2, but it did show modest efficacy against HSV-1 infection and symptomatic HSV-1 genital disease. Participants in this trial did not demonstrate evidence of neutralizing antibodies 16 months following vaccination. It is unclear why the initial trial demonstrated a sex difference in protection against HSV-2 that was not replicated in the second trial.

11.3 Behavioral and Biological Bases for Sex Differences in RTIs

11.3.1 Behavioral

Sexually transmitted infections spread through social networks. For many infections, individual-level risk factors such as the number of lifetime sexual partners or condom use only partially explain why some populations are afflicted by these infections. Consequently, network-level factors are likely responsible for explaining some of the remaining differences. Social network theory posits that an individual's social network, including the number, type, and content of network ties, affects an individual's health-related behaviors and risk of negative health outcomes (Black et al. 2013). For RTIs, sexual network-level factors, such as the proportion of partnerships that run concurrently, may also provide a plausible explanation. There have been differences noted in networks between the sexes. A recent study found that gender differences in social networks may partially explain gender differences in STI risk for homeless youths in San Francisco (Valente and Auerswald 2013). Young women in that study were less likely to use condoms and

were more likely to have an injection drug-using partner. Young women were also less likely to have stably housed network contacts than young men. Concurrency of partners has a major impact on enhancing STI transmission by increasing a sexual network's connectivity (Robinson et al. 2012). For example, an ecological study found an association between prevalence of point concurrency and female HSV-2 prevalence between different countries (Kenyon et al. 2013).

11.3.2 Biological

There are several biological factors that may explain sex differences observed in the susceptibility to and the pathophysiology of RTIs. Genetic differences between the sexes may impact immune responses and other biological processes such as drug absorption and metabolism (see Chap. 4). In this section, we will focus on three biological factors: anatomical differences, differences in sex hormones, and the unique role of the vaginal microbiome in health and disease states.

The female reproductive tract is a complex system where immune cells, hormones, and microorganisms interact (Fig. 11.1). It may be divided into two parts: the lower (vagina and ectocervix) and upper (endocervix, uterus, fallopian tubes) tracts. Multiple layers of stratified squamous epithelial cells that lack tight junctions

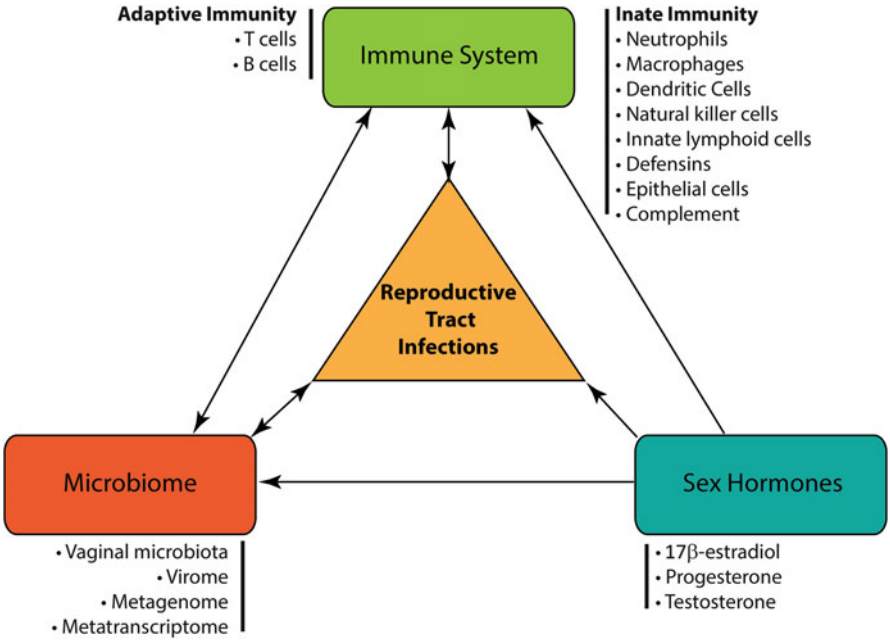

Fig. 11.1 There are multiple potential interactions between the immune system, sex hormones, the microbiome, and RTIs. Some of these interactions may be bidirectional

line the lower tract. The upper tract epithelium consists of a single tightly bound layer of columnar cells. The transition or transformation zone between the two is highly immunologically active.

11.3.2.1 Anatomical

Female anatomy makes transmission of infections to women more efficient than to males. The supine position allows secretions deposited during intercourse to pool in the vaginal vault, where they bathe the uterine cervix. Although this is biologically advantageous for procreation by providing spermatozoa prolonged access to the female reproductive tract, it also increases the intensity and duration of exposure to infection if the woman's male sexual partner has an infection (Hook 2012).

Age- and hormone-related changes in the amount of endocervical columnar epithelial cells exposed around the cervical transition zone (referred to as cervical ectopy) are another anatomical factor that increases the risk of infection in women (Harrison et al. 1985). Cervical ectopy results in a greater number of potential "target" cells for infection if exposed to infectious agents such as NG. The prevalence of cervical ectopy ranges from 17 to 50 % (Goldacre et al. 1978). It is more common among adolescents, pregnant women, and women using hormonal contraceptives. A recent study reported a higher concentration of inflammatory cells and regulatory cytokines and chemokines in ectopic zones (Moscicki et al. 2001). Observational studies have linked cervical ectopy to the increased acquisition of some RTIs including chlamydia and human papillomavirus (HPV) (Jacobson et al. 2000). Anatomic changes as a result of endogenous hormonal cycles in women (i.e., menstrual cycles) are also associated with risk of infection. For example, several reports beginning in the 1950s suggested that there was an increased risk of diagnosing NG in women shortly after the beginning of menstruation (Putkonen and Ebeling 1950) and an increased risk of DGI around that time. Several hypotheses were suggested: menstrual blood providing nutritional supplementation enhancing the growth of the organism, hormonally mediated increased adherence of the organism, and favorable characteristics of the cervical mucus. In monkeys, exogenous progesterone results in thinning of the epithelial layer resulting in enhanced susceptibility to simian immunodeficiency virus (Poonia et al. 2006). Studies in humans, however, have not reproduced these findings (Mauck et al. 1999; Bahamondes et al. 2000).

11.3.2.2 Sex Hormones

Impact on Mucosal Immune Responses of the Reproductive Tract

Immune responses in the female genital tract are regulated by sex hormones (Paavonen 1994). Antigen presentation, cytokine production, immunoglobulin production and transport, and even induction of tolerance are influenced by

variations in sex hormone levels (Prabhala and Wira 1995; Wira and Rossoll 1995; Wira et al. 2000; Black et al. 2000). The interaction between sex hormones and the immune system is complex, and the variation of hormonal effects between species further complicates extrapolation of data from murine models to humans. In autoimmune diseases, estrogens have been shown to have biphasic effects, with low levels enhancing and high levels inhibiting relapses of autoimmune diseases, including multiple sclerosis (Calabrese 2001). Testosterone is more consistently anti-inflammatory (Paavonen 1994). Others have shown that low estrogen levels favor the generation of a T_H1 response, whereas high estrogen and testosterone levels favor a T_H2 response (Faas et al. 2000). Studies of cervicovaginal immune responses in women suggest that there is a dampening in cervical immune responses around the time of ovulation. This is consistent with the body's attempt to optimize the environment to promote successful fertilization and subsequent embryo development. Some investigators have defined the term "window of vulnerability" that begins shortly before ovulation (i.e., around day 12 of a normal menstrual cycle, the preovulatory follicular phase at the time of the estradiol peak) and persists until around day 21 (i.e., mid-luteal phase around the time of the progesterone peak) (Hel et al. 2010). The use of exogenous sex hormones, i.e., hormonal contraception, by hundreds of millions of women worldwide, further complicates the picture and requires additional investigation.

Impact on Reproductive Tract Infections

Observational studies have suggested a relationship between menstrual cycle phase and/or hormonal contraceptive use and risk of RTIs. The time elapsed from the onset of menses was found to be an independent risk factor for *Chlamydia trachomatis* infection of the cervix (Rosenthal and Landefeld 1990). PID caused by both chlamydia and NG occurred more frequently within 1 week after the onset of menses. A prospective study of Kenyan commercial sex workers showed an increased risk for acquisition of cervicitis and vaginal candidiasis and a decreased risk for trichomoniasis and PID in women using long-acting depot medroxyprogesterone acetate (DMPA) (Baeten et al. 2001). Another study revealed higher prevalence of CT in the lower genital tract and increased incidence of silent endometritis and salpingitis in oral contraceptive users (Wolner-Hanssen et al. 1990).

Several prospective studies were conducted to evaluate the risk of gonococcal cervicitis in hormonal contraceptive users. In a study of 818 women recruited from sexually transmitted disease clinics in Birmingham, Alabama, hormonal contraception increased the risk of gonococcal cervicitis by 70 % (Louv et al. 1989) compared to age-matched women not taking hormonal contraceptives. In contrast, another study of 948 Kenyan commercial sex workers did not reveal an increased risk of gonococcal infection among hormonal contraceptive users (either depot medroxyprogesterone acetate or combined oral contraceptive pills) (Baeten et al. 2001). Finally, a prospective study of 819 women in Baltimore, MD, who

were followed for 1 year revealed that only depot medroxyprogesterone acetate was significantly associated with a nearly fourfold increased risk of both chlamydial and gonococcal cervical infections (Morrison et al. 2004) compared to nonusers. There are no convincing data that the risk of gonorrhea during pregnancy is altered. A significant limitation of studies assessing hormonal contraception and the risk of RTIs is the possible coexistence of behavioral confounding. Women who take hormonal contraception may be less likely to use condoms with their male sexual partners because they are using hormonal contraception to prevent unwanted pregnancies. As most epidemiological studies assessing the role of hormonal contraception have been observational studies, residual confounding cannot be ruled out and the association between hormonal contraception and RTIs may not be causative.

Sex hormones appear to play an important role in HSV pathogenesis. Most studies evaluating the effects of sex hormones on HSV susceptibility and progression have focused on HSV-2. In a cross-sectional study of 273 women who were seropositive for HSV-2, women using either DMPA or combined oral contraceptives were more likely to experience HSV-2 shedding (Mostad et al. 1997). A longitudinal study of women initiating hormonal contraception, however, did not confirm this association (Wang et al. 2004). One study demonstrated a nearly eightfold increased odds of genital HSV-DNA shedding among pregnant women (Mostad et al. 2000). Taken together these data suggest that sustained or elevated concentrations of sex hormones may increase HSV-2 shedding, but additional studies are required to confirm this association in both pregnant and nonpregnant women.

The data on hormonal contraception and HSV-2 acquisition are much more limited. In a study of 302 Kenyan commercial sex workers, hormonal contraception increased the risk of genital ulcer disease acquisition, with HSV-2 responsible for only 50 % of the genital ulcers (Baeten et al. 2007). In a prospective study of 948 Kenyan commercial sex workers, neither DMPA nor combined oral contraceptive use increased incident genital ulcer disease acquisition (Baeten et al. 2001). Additional prospective studies focusing on HSV are needed to assess whether an association exists between hormonal contraceptives and HSV acquisition.

Significant data on the effect of sex hormones on susceptibility to and the course of HSV-2 infection have been obtained from mouse models. In one study, ovariectomized mice were administered estradiol, progesterone, a combination of both hormones, or vehicle (Gillgrass et al. 2005b). The vehicle-treated and progesterone-treated mice were more susceptible to HSV-2 infection with extensive infection of the vaginal epithelium within 24 h after infection and significant induction of inflammatory chemokine and chemokine receptors. In contrast, estradiol-treated mice were protected from HSV-2 infection. A slower progression of genital pathology was noted in the group receiving combination hormones as compared to the vehicle-treated mice. The same investigators immunized mice with an attenuated HSV-2 vaccine following either estradiol or progesterone injections and then challenged them with a virulent HSV-2 strain (Gillgrass et al. 2005a). Mice injected with progesterone were protected from subsequent rechallenge, whereas estradiol-

treated mice were not. These findings suggested that protection against challenge was dependent upon the ability of the attenuated strain to cause infection, which, in turn, was dependent upon the hormonal environment. Finally, these investigators showed that duration of exposure to hormones had a significant effect on responses, in which mice that were exposed to progesterone for 5 days and immunized with an attenuated HSV-2 vaccine were protected from rechallenge with a virulent strain, but those exposed to progesterone for 15 days prior to immunization were not (Gillgrass et al. 2005a). These data suggest that the hormonal milieu as well as duration of exposure significantly affect the efficacy of vaccines and susceptibility to HSV infection.

Perhaps the most intriguing association between sex hormones and HSV-2 was the data from the glycoprotein-D-subunit vaccine which, overall, was not found to be protective against acquisition of HSV infection (Stanberry et al. 2002). However, in subgroup analyses, women who were initially seronegative for both HSV-1 and HSV-2 were protected against incident herpes infection after receiving the vaccine but no similar association was noted in men. It was hypothesized that hormonal differences between men and women may be responsible for these findings. A subsequent animal study confirmed the biological plausibility of this hypothesis. Investigators used the vaccine in a mouse model and demonstrated that estradiol improved vaccine-elicited protection against genital herpes infection and resulted in an enhanced antibody response (Pennock et al. 2009).

11.3.2.3 Vaginal Microbiome

The bacterial communities that naturally colonize the vagina (termed the "vaginal microbiota") present another dramatic difference in how men and women vary in their defenses against infection. The vaginal microbiota play an important role in preventing colonization by pathogenic organisms, including sexually transmitted infections and urinary tract infectious agents (Cohen et al. 2012; Brotman et al. 2010; Martin 2012; Peipert et al. 2008; Cherpes et al. 2003a, b; Martin et al. 1999; King et al. 2011; Myer et al. 2005a; Gallo et al. 2012; Balkus et al. 2014; Ghartey et al. 2014; Phukan et al. 2013). The vaginal microbiome provides protection in part through the influential action of *Lactobacillus* spp. Some vaginal *Lactobacillus* spp. are known to provide broad-spectrum defense through their production of copious amounts of lactic acid (Boskey et al. 2001), bacteriocins (bactericidal proteinaceous molecules) (Aroutcheva et al. 2001; Martin et al. 1999; Myer et al. 2005b; Ness et al. 2005; Peipert et al. 2008), antagonistic bacteriocin-like substances (Ocana et al. 1999), and biosurfactants (Reid et al. 1999) and through their ability to adhere to mucus, which enable them to form a barrier against pathogens (Boris and Barbés 2000) and disrupt biofilms (McMillan et al. 2011). Hydrogen peroxide is produced by many *Lactobacillus* species (but notably not all *L. iners* (Vallor et al. 2001; Hillier et al. 1993; Hawes et al. 1996)) and has been thought to have wide-spectrum antimicrobial activity. However, a recent study shows lactic acid, and not hydrogen peroxide, was the dominant

antimicrobial agent (O'Hanlon et al. 2011). There is also evidence that lactic acid possesses antimicrobial activity beyond acidity alone by controlling overgrowth of bacteria and disrupting the integrity of some bacterial cell membranes (Aldunate et al. 2013; Graver and Wade 2011; Lai et al. 2009; O'Hanlon et al. 2011; Motevaseli et al. 2013; Alakomi et al. 2000). Further, recent work has demonstrated that lactobacilli influence the ability of *Trichomonas vaginalis* to adhere to host cells, thereby altering the virulence of this parasite (Phukan et al. 2013).

Disruptions of the vaginal microbiota can result in the clinical diagnosis of BV. BV is the most cited cause of vaginal symptoms (primarily malodor and discharge) prompting women to present to primary health care (Amsel et al. 1983; Sobel 2005). BV is characterized by a relatively low abundance of protective *Lactobacillus* spp. and overgrowth of diverse anaerobes including *Gardnerella vaginalis*, *Prevotella* spp., *Mobiluncus* spp., *Atopobium vaginae* as well as other taxa of the order *Clostridiales* (Fredricks et al. 2005). With prevalence rates for BV ranging between 29 % of US women (Koumans et al. 2007) and 50 % of women in rural Ugandan villages (Wawer et al. 1999), BV is a major population-level risk factor for RTIs which has largely been overlooked (Brotman 2011). Antibiotic treatment for BV results in high recurrence rates owing in part to our deficiencies in understanding the etiologies involved with the disruptions in the vaginal microbiome (Sobel et al. 2006).

The overwhelming majority of microbial species (>90 %) resist cultivation in the laboratory (Bakken 1985), and as a result, we have had an incomplete understanding of the microbes which inhabit the human body. The recent development of next-generation sequencing technologies has revolutionized how we characterize the human microbiome, as it has enabled sequencing hundreds of thousands of bacteria in a quantitative and affordable way (Hugenholtz et al. 1998; Torsvik and Ovreas 2002). It is becoming the standard to perform massively parallel sequencing of 16S rRNA gene amplicons to produce thousands of sequences per sample.

Cultivation-independent studies based on the analysis of 16S rRNA gene sequences amplified from whole genomic DNA isolated from vaginal samples have dramatically changed our understanding of vaginal microbial community diversity and have identified bacteria that could not be observed by traditional culture-based methods (Fredricks et al. 2005; Ferris et al. 2004; Ravel et al. 2011). Overall, molecular studies have shown the diversity, composition, and relative abundance of microbial species in the vagina vary dramatically between women. In the first study of its kind, analyzing 394 women in the United States from four different ethnicities, Ravel et al. used 16S rRNA gene analysis to identify five major groupings (termed "community state types") of vaginal microbiota in reproductive-age women (Ravel et al. 2011). Four of these groups were dominated by a species of *Lactobacillus* (*L. crispatus*, *L. iners*, *L. gasseri*, *L. jensenni*), while the fifth group was depleted of *Lactobacillus* (Fig. 11.2). The latter group contained higher proportions of anaerobic bacteria, resembling BV and work is ongoing to further refine how this community state type is categorized and evaluated (Brotman et al. 2014a). The frequencies of each bacterial state vary greatly by ethnicity, and African American and Hispanic women are more likely to be *Lactobacillus* depleted

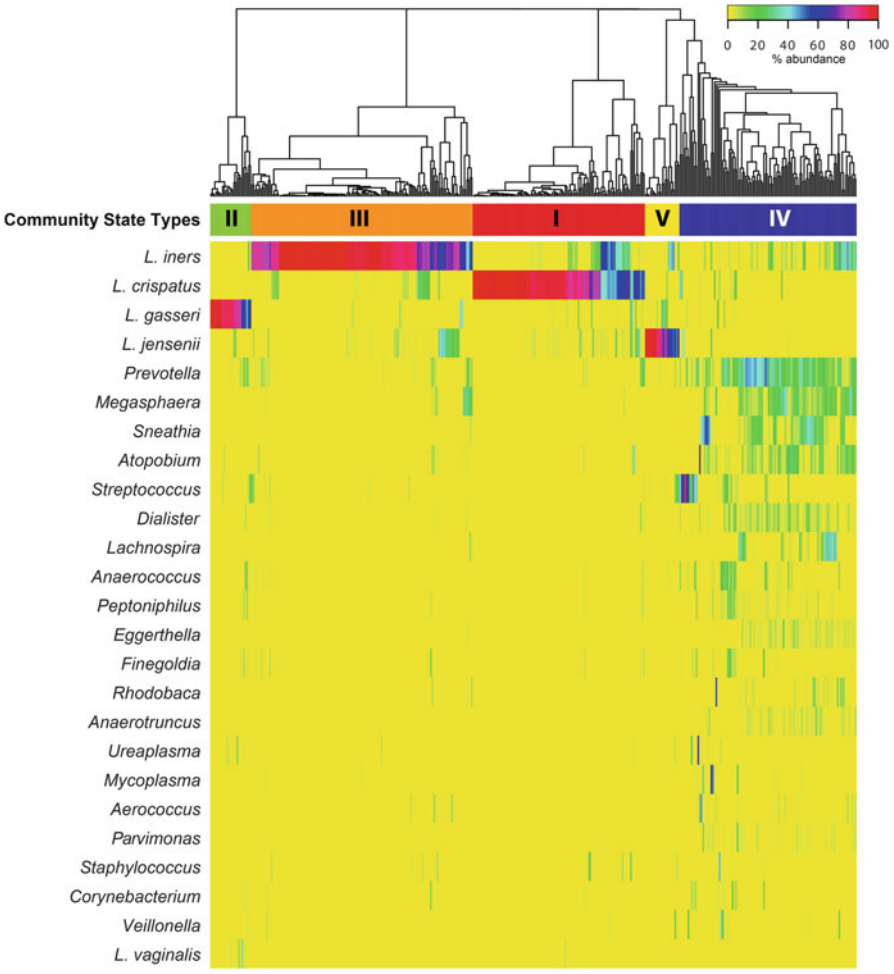

Fig. 11.2 Heatmap showing the distribution of bacterial taxa found in the vaginal microbial communities of 394 reproductive-age women [Adapted with permission from *Proceedings of the National Academy of Sciences of the United States of America* (Ravel et al. 2011)]

(Ravel et al. 2011). The reasons for racial disparity in the vaginal microbiome are unknown and likely play a role in the observation that African American women and Hispanic women have higher prevalence of RTIs (Datta et al. 2007; Miller et al. 2004).

Prospective longitudinal studies with frequent sampling add tremendous information to what we currently understand about the dynamics of the vaginal microbiota. Some women experience frequent and rapid fluctuations in the composition of the vaginal microbiota, while in others, the microbiota are remarkably stable (Ravel et al. 2013; Gajer et al. 2012; Srinivasan et al. 2010). Epidemiologic studies have demonstrated that fluctuations in the vaginal microbiota are mainly

affected by time in the menstrual cycle and sexual activity (Gajer et al. 2012; Keane et al. 1997; Bradshaw et al. 2013; Srinivasan et al. 2010). Figure 11.3 illustrates the dynamics of the vaginal microbiota observed among reproductive-age women (Ravel et al. 2013). The figure depicts six women who self-collected mid-vaginal

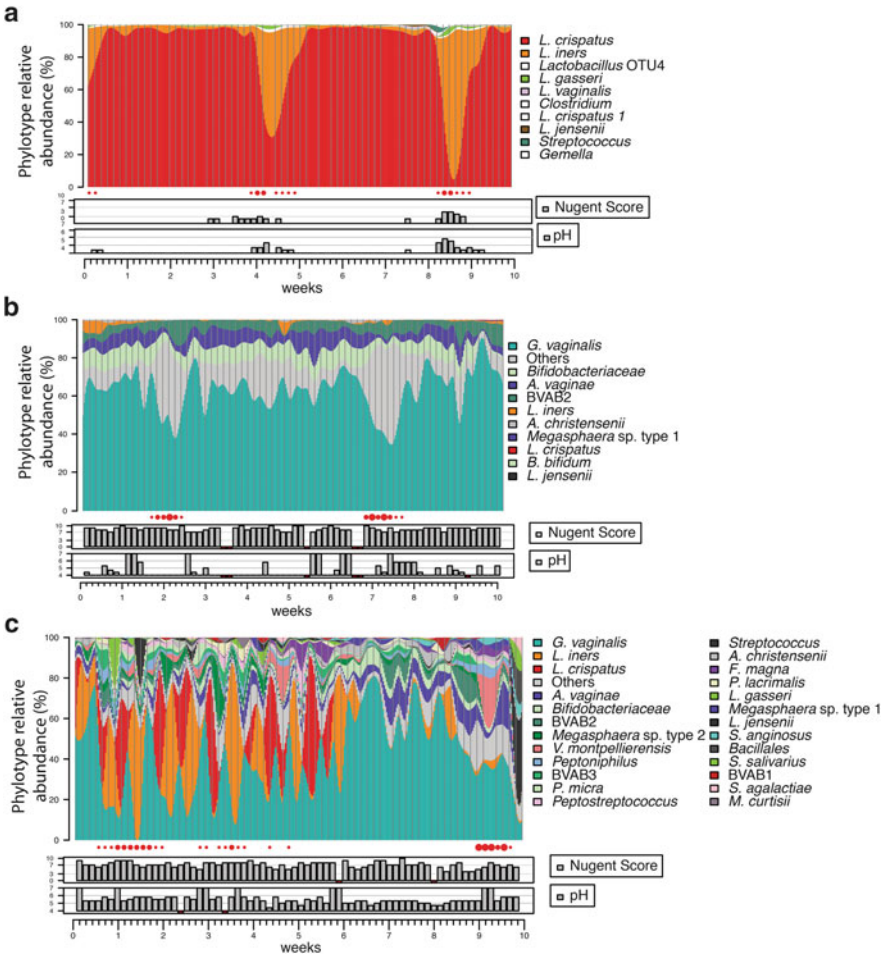

Fig. 11.3 Daily temporal dynamics of vaginal bacterial communities in 3 women over a 10-week period. The relative abundance of each phylotype is depicted as interpolated bar graphs. Phlyotype *color* codes are indicated on the *right* of each bar graph. Daily Nugent Gram stain scores (range 0-10) and pH (range 4-7) are indicated below the graph. *Red solid circles* represent menstruation. Missing pH values are indicated by *red box*; otherwise, pH is in line with a value of 4. Missing Nugent scores are also indicated by the *red box*; otherwise, the score is in line with 0. The figures shows that the top two participants (**a, b**) carried highly stable communities dominated by *L. crispatus* (**a**) and non-*Lactobacillus* dominated communities (**b**). Woman (**c**) experienced very low stability communities with both high Nugent scores and pH. [Adapted with permission from *Microbiome* (Ravel et al. 2013).

samples daily for 10 weeks and the change in relative abundance of the most abundant bacterial taxa over time. The relative abundance of each bacterium is color coded according to the key. Although only the top 10–20 bacterial taxa are displayed in each panel, over 265 taxa have been identified in the vaginal microbiome (Ravel et al. 2011). The woman displayed in **panel a** has a vaginal microbiome that is dominated by *L. crispatus* except for brief transitions to a *L. iners*-dominated state during the intervals of menstrual bleeding. Similarly, the woman displayed in **panel b** is consistently stable in a *L. iners*-dominated profile except for intervals during menstruation when *Streptococcus* spp. emerged. The women in **panels c and d** are relatively stable over time in profiles with low relative abundance of *Lactobacillus* spp., while the women displayed in **panels e and f** also have low-*Lactobacillus* profiles but have rapid fluctuations in bacterial communities.

Although the clinical relevance of the rapid fluctuation in vaginal microbiota is still not completely understood, there are emerging data that link these fluctuations to RTI susceptibility (Buvé et al. 2014; Brotman 2011; Martin et al. 2013). In a longitudinal study of 32 women who self-collected samples twice weekly for 16 weeks (Brotman et al. 2014b), several vaginal microbiota community state types were associated with changes in HPV status. *L. gasseri*-dominated community state types had the fastest HPV remission rate and a low-*Lactobacillus* community with high proportions of the genera *Atopobium* (CST IV-B) had the slowest rate compared to *L. crispatus*-dominated community state types (adjusted transition rate ratio (aTRR), 4.43, 95 % CI, 1.11–17.7; aTRR, 0.33, 95 % CI, 0.12–1.19, respectively). This data suggests the vaginal microbiome may play a significant role in the control of latent HPV infection or increased clearance of HPV. Longitudinal studies on the incidence and clearance of RTIs are now beginning to include molecular assessments of the vaginal microbiome and will increase the understanding of how the vaginal microbiome protects against RTIs.

Vaginal Microbiome, Sex Hormones, and Mucosal Immune Responses

A key influence on the vaginal microbiome is endogenous estrogens (Hillier and Lau 1997; Hummelen et al. 2011). During a woman's reproductive years, estrogens stimulate the proliferation of vaginal epithelial cells, promoting a rich glycogen environment that provides an affluent source for fermentative microbes to metabolize. The acidic vaginal environment is maintained by lactic acid and other organic acids through colonized microorganisms (i.e., the protective *Lactobacillus* spp.) metabolizing glycogen in the vaginal mucosa (Cruickshank 1934; Jakobsson and Forsum 2008).

With menopause and declining concentrations of estrogens, studies have demonstrated a lower relative abundance of vaginal *Lactobacillus* sp. (Hillier and Lau 1997; Pabich et al. 2003; Brotman et al. 2014a) and greater abundance of vaginal *Escherichia coli* and enterococci (Pabich et al. 2003). The association between estrogens and the vaginal microbiome is also supported by studies that demonstrate

that hormone replacement therapy restores lactobacilli in vaginal microbiota of menopausal women (Devillard et al. 2004; Heinemann and Reid 2005).

The immune system has also coevolved with microbes to maintain a homeostatic host-microbial relationship (Hooper et al. 2012). The vaginal microbiota stimulate the local innate immune system as part of its protective mechanisms in the female genital tract (Mirmonsef et al. 2011; Witkin et al. 2007). A disrupted vaginal microbiota may lead to clinical or subclinical mucosal inflammatory response, changes in innate mucosal immunity, microtraumas to the vaginal epithelium, or puncturing of the cervicovaginal mucosa. The low-*Lactobacillus* state of BV has been associated with genital pro-inflammatory cytokine upregulation, although some studies have found that downregulation of some cytokines also occurs (Yudin et al. 2003; Sturm-Ramirez et al. 2000; Ryckman et al. 2008).

The local cytokine production associated with a disrupted vaginal microbiota may facilitate the acquisition of RTIs. Inflammation is an immune process essential for microbial control and clearance that is initiated and sustained by cytokine and chemokine production in response to pathogen recognition (Svanborg et al. 1999). RTIs are the major causes of inflammatory cytokine upregulation and immune cell recruitment to the genital mucosa (Fichorova et al. 2001; Levine et al. 1998; Yudin et al. 2003; Reddy et al. 2004). Although inflammation can play an important role in infection clearance, it may also cause destruction of infected epithelial layers, allowing pathogens to access deeper tissues (Svanborg et al. 1999; McGee et al. 1999).

11.4 Conclusion

Because of greater biological susceptibilities due in part to differences in anatomy, sex hormone concentrations, and the microbiome, women have a higher incidence of several RTIs than men. Additionally, women are less frequently symptomatic than men when infected. Consequently, RTIs may go undiagnosed and untreated in women and lead to severe health consequences, such as PID, infertility, and increased HIV risk. It is now recognized that the vaginal microbiome plays a major role in women's reproductive health, with between 30 and 50 % of women lacking a microbiota that can provide optimal protection against pathogens. Further research is needed to better define the interactions among the immune system, sex hormones, and the vaginal microbiome with the goal of enhancing women's reproductive health.

References

Alakomi HL, Skytta E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM (2000) Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Appl Environ Microbiol 66:2001–2005

Aldunate M, Tyssen D, Johnson A, Zakir T, Sonza S, Moench T, Cone R, Tachedjian G (2013) Vaginal concentrations of lactic acid potently inactivate HIV. J Antimicrob Chemother 68:2015–2025

Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK (1983) Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med 74:14–22

Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, Gurguis A, Faro S (2001) Defense factors of vaginal lactobacilli. Am J Obstet Gynecol 185:375–379

Baeten JM, Nyange PM, Richardson BA, Lavreys L, Chohan B, Martin HL Jr, Mandaliya K, Ndinya-Achola JO, Bwayo JJ, Kreiss JK (2001) Hormonal contraception and risk of sexually transmitted disease acquisition: results from a prospective study. Am J Obstet Gynecol 185:380–385

Baeten JM, Benki S, Chohan V, Lavreys L, Mcclelland RS, Mandaliya K, Ndinya-Achola JO, Jaoko W, Overbaugh J (2007) Hormonal contraceptive use, herpes simplex virus infection, and risk of HIV-1 acquisition among Kenyan women. AIDS 21:1771–1777

Bahamondes L, Trevisan M, Andrade L, Marchi NM, Castro S, Diaz J, Faundes A (2000) The effect upon the human vaginal histology of the long-term use of the injectable contraceptive Depo-Provera. Contraception 62:23–27

Bakken LR (1985) Separation and purification of bacteria from soil. Appl Environ Microbiol 49:1482–1487

Balkus JE, Richardson BA, Rabe LK, Taha TE, Mgodi N, Kasaro MP, Ramjee G, Hoffman IF, Abdool Karim SS (2014) Bacterial vaginosis and the risk of trichomonas vaginalis acquisition among HIV-1–negative women. Sex Transm Dis 41:123–128

Belkacem A, Caumes E, Ouanich J, Jarlier V, Dellion S, Cazenave B, Goursaud R, Lacassin F, Breuil J, Patey O, Working Group F-D (2013) Changing patterns of disseminated gonococcal infection in France: cross-sectional data 2009–2011. Sex Transm Infect 89:613–615

Belshe RB, Leone PA, Bernstein DI, Wald A, Levin MJ, Stapleton JT, Gorfinkel I, Morrow RLA, Ewell MG, Stokes-Riner A, Dubin G, Heineman TC, Schulte JM, Deal CD, Herpevac Trial for Women (2012) Efficacy results of a trial of a herpes simplex vaccine. N Engl J Med 366:34–43

Bernstein DI, Bellamy AR, Hook EW, Levin MJ, Wald A, Ewell MG, Wolff PA, Deal CD, Heineman TC, Dubin G, Belshe RB (2013) Epidemiology, clinical presentation, and antibody response to primary infection with herpes simplex virus type 1 and type 2 in young women. Clin Infect Dis 56:344–351

Black CA, Rohan LC, Cost M, Watkins SC, Draviam R, Alber S, Edwards RP (2000) Vaginal mucosa serves as an inductive site for tolerance. J Immunol 165:5077–5083

Black SR, Schmiege S, Bull S (2013) Actual versus perceived peer sexual risk behavior in online youth social networks. Transl Behav Med 3:312–319

Boris S, Barbés C (2000) Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect 2:543–546

Boskey ER, Cone RA, Whaley KJ, Moench TR (2001) Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. Hum Reprod 16:1809–1813

Bradshaw CS, Walker J, Fairley CK, Chen MY, Tabrizi SN, Donovan B, Kaldor JM, Mcnamee K, Urban E, Walker S, Currie M, Birden H, Bowden F, Garland S, Pirotta M, Gurrin L, Hocking JS (2013) Prevalent and incident bacterial vaginosis are associated with sexual and contraceptive behaviours in young Australian women. PLoS One 8:e57688

Britigan BE, Cohen MS, Sparling PF (1985) Gonococcal infection: a model of molecular pathogenesis. N Engl J Med 312:1683–1694

Brotman RM (2011) Vaginal microbiome and sexually transmitted infections: an epidemiologic perspective. J Clin Invest 121:4610–4617

Brotman RM, Klebanoff MA, Nansel TR, Yu KF, Andrews WW, Zhang J, Schwebke JR (2010) Bacterial vaginosis assessed by gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. J Infect Dis 202:1907–1915

Brotman RM, Shardell MD, Gajer P, Fadrosh D, Chang K, Silver MI, Viscidi RP, Burke AE, Ravel J, Gravitt PE (2014a) Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. Menopause 21:450–458

Brotman RM, Shardell MD, Gajer P, Tracy JK, Zenilman JM, Ravel J, Gravitt PE (2014b) Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. J Infect Dis 210:1723–1733

Bryson Y, Dillon M, Bernstein DI, Radolf J, Zakowski P, Garratty E (1993) Risk of acquisition of genital herpes simplex virus type 2 in sex partners of persons with genital herpes: a prospective couple study. J Infect Dis 167:942–946

Buvé A, Jespers V, Crucitti T, Fichorova RN (2014) The vaginal microbiota and susceptibility to HIV. AIDS 28:2333–2344

Calabrese EJ (2001) Androgens: biphasic dose responses. Crit Rev Toxicol 31:517–522

Cherpes TL, Meyn LA, Krohn MA, Hillier SL (2003a) Risk factors for infection with herpes simplex virus type 2: role of smoking, douching, uncircumcised males, and vaginal flora. Sex Transm Dis 30:405–410

Cherpes TL, Meyn LA, Krohn MA, Lurie JG, Hillier SL (2003b) Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. Clin Infect Dis 37:319–325

Cohen CR, Lingappa JR, Baeten JM, Ngayo MO, Spiegel CA, Hong T, Donnell D, Celum C, Kapiga S, Delany S, Bukusi EA (2012) Bacterial vaginosis associated with increased risk of female-to-male HIV-1 transmission: a prospective cohort analysis among African couples. PLoS Med 9:e1001251

Corey L, Wald A, Patel R, Sacks SL, Tyring SK, Warren T, Douglas JM, Paavonen J, Morrow RA, Beutner KR, Stratchounsky LS, Mertz G, Keene ON, Watson HA, Tait D, Vargas-Cortes M, Valacyclovir HSV Transmission Study Group (2004) Once-daily valacyclovir to reduce the risk of transmission of genital herpes. N Engl J Med 350:11–20

Cruickshank R (1934) The conversion of the glycogen of the vagina into lactic acid. J Pathol Bacteriol 39:213–219

Datta SD, Sternberg M, Johnson RE, Berman S, Papp JR, Mcquillan G, Weinstock H (2007) Gonorrhea and chlamydia in the United States among persons 14 to 39 years of age, 1999 to 2002. Ann Intern Med 147:89–96

Devillard E, Burton JP, Hammond JA, Lam D, Reid G (2004) Novel insight into the vaginal microflora in postmenopausal women under hormone replacement therapy as analyzed by PCR-denaturing gradient gel electrophoresis. Eur J Obstet Gynecol Reprod Biol 117:76–81

Division of STD Prevention, C D C (2013) Sexually transmitted disease surveillance 2012, pp 1–174

Faas M, Bouman A, Moesa H, Heineman MJ, De Leij L, Schuiling G (2000) The immune response during the luteal phase of the ovarian cycle: a Th2-type response? Fertil Steril 74:1008–1013

Ferris MJ, Masztal A, Martin DH (2004) Use of species-directed 16S rRNA gene PCR primers for detection of Atopobium vaginae in patients with bacterial vaginosis. J Clin Microbiol 42:5892–5894

Fichorova RN, Desai PJ, Gibson FC 3rd, Genco CA (2001) Distinct proinflammatory host responses to Neisseria gonorrhoeae infection in immortalized human cervical and vaginal epithelial cells. Infect Immun 69:5840–5848

Fredricks DN, Fiedler TL, Marrazzo JM (2005) Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med 353:1899–1911

Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ (2006) Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. AIDS 20:73–83

Gajer P, Brotman RM, Bai G, Sakamoto J, Schutte UM, Zhong X, Koenig SS, Fu L, Ma ZS, Zhou X, Abdo Z, Forney LJ, Ravel J (2012) Temporal dynamics of the human vaginal microbiota. Sci Transl Med 4:132ra52

Gallo MF, Macaluso M, Warner L, Fleenor ME, Hook EW 3rd, Brill I, Weaver MA (2012) Bacterial vaginosis, gonorrhea, and chlamydial infection among women attending a sexually

transmitted disease clinic: a longitudinal analysis of possible causal links. Ann Epidemiol 22:213–220
Ghartey JP, Smith BC, Chen Z, Buckley N, Lo Y, Ratner AJ, Herold BC, Burk RD (2014) Lactobacillus crispatus dominant vaginal microbiome is associated with inhibitory activity of female genital tract secretions against Escherichia coli. PLoS One 9:e96659
Gillgrass AE, Fernandez SA, Rosenthal KL, Kaushic C (2005a) Estradiol regulates susceptibility following primary exposure to genital herpes simplex virus type 2, while progesterone induces inflammation. J Virol 79:3107–3116
Gillgrass AE, Tang VA, Towarnicki KM, Rosenthal KL, Kaushic C (2005b) Protection against genital herpes infection in mice immunized under different hormonal conditions correlates with induction of vagina-associated lymphoid tissue. J Virol 79:3117–3126
Goldacre MJ, Loudon N, Watt B, Grant G, Loudon JD, Mcpherson K, Vessey MP (1978) Epidemiology and clinical significance of cervical erosion in women attending a family planning clinic. Br Med J 1:748–750
Graver MA, Wade JJ (2011) The role of acidification in the inhibition of Neisseria gonorrhoeae by vaginal lactobacilli during anaerobic growth. Ann Clin Microbiol Antimicrob 10:8
Handsfield HH (1975) Disseminated gonococcal infection. Clin Obstet Gynecol 18:131–142
Harrison HR, Costin M, Meder JB, Bownds LM, Sim DA, Lewis M, Alexander ER (1985) Cervical Chlamydia trachomatis infection in university women: relationship to history, contraception, ectopy, and cervicitis. Am J Obstet Gynecol 153:244–251
Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wolner-Hanssen P, Holmes KK (1996) Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. J Infect Dis 174:1058–1063
Heinemann C, Reid G (2005) Vaginal microbial diversity among postmenopausal women with and without hormone replacement therapy. Can J Microbiol 51:777–781
Hel Z, Stringer E, Mestecky J (2010) Sex steroid hormones, hormonal contraception, and the immunobiology of human immunodeficiency virus-1 infection. Endocr Rev 31:79–97
Hillier SL, Lau RJ (1997) Vaginal microflora in postmenopausal women who have not received estrogen replacement therapy. Clin Infect Dis 25(Suppl 2):S123–S126
Hillier SL, Krohn MA, Rabe LK, Klebanoff SJ, Eschenbach DA (1993) The normal vaginal flora, H2O2-producing lactobacilli, and bacterial vaginosis in pregnant women. Clin Infect Dis 16 (Suppl 4):S273–S281
Hook EW (2012) Gender differences in risk for sexually transmitted diseases. Am J Med Sci 343:10–11
Hooper LV, Littman DR, Macpherson AJ (2012) Interactions between the microbiota and the immune system. Science 336:1268–1273
Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J Bacteriol 180:4765–4774
Hummelen R, Macklaim JM, Bisanz JE, Hammond JA, Mcmillan A, Vongsa R, Koenig D, Gloor GB, Reid G (2011) Vaginal microbiome and epithelial gene array in post-menopausal women with moderate to severe dryness. PLoS One 6:e26602
Jacobson DL, Peralta L, Farmer M, Graham NM, Gaydos C, Zenilman J (2000) Relationship of hormonal contraception and cervical ectopy as measured by computerized planimetry to chlamydial infection in adolescents. Sex Transm Dis 27:313–319
Jakobsson T, Forsum U (2008) Changes in the predominant human Lactobacillus flora during in vitro fertilisation. Ann Clin Microbiol Antimicrob 7:14
Johnson HL, Ghanem KG, Zenilman JM, Erbelding EJ (2011) Sexually transmitted infections and adverse pregnancy outcomes among women attending inner city public sexually transmitted diseases clinics. Sex Transm Dis 38:167–171
Keane FE, Ison CA, Taylor-Robinson D (1997) A longitudinal study of the vaginal flora over a menstrual cycle. Int J STD AIDS 8:489–494
Kenyon C, Colebunders R, Hens N (2013) Determinants of generalized herpes simplex virus-2 epidemics: the role of sexual partner concurrency. Int J STD AIDS 24:375–382

King CC, Jamieson DJ, Wiener J, Cu-Uvin S, Klein RS, Rompalo AM, Shah KV, Sobel JD (2011) Bacterial vaginosis and the natural history of human papillomavirus. Infect Dis Obstet Gynecol 2011:319460

Koumans EH, Sternberg M, Bruce C, Mcquillan G, Kendrick J, Sutton M, Markowitz LE (2007) The prevalence of bacterial vaginosis in the United States, 2001–2004; associations with symptoms, sexual behaviors, and reproductive health. Sex Transm Dis 34:864–869

Lai SK, Hida K, Shukair S, Wang YY, Figueiredo A, Cone R, Hope TJ, Hanes J (2009) Human immunodeficiency virus type 1 is trapped by acidic but not by neutralized human cervicovaginal mucus. J Virol 83:11196–11200

Levine WC, Pope V, Bhoomkar A, Tambe P, Lewis JS, Zaidi AA, Farshy CE, Mitchell S, Talkington DF (1998) Increase in endocervical CD4 lymphocytes among women with nonulcerative sexually transmitted diseases. J Infect Dis 177:167–174

Liu B, Roberts CL, Clarke M, Jorm L, Hunt J, Ward J (2013) Chlamydia and gonorrhoea infections and the risk of adverse obstetric outcomes: a retrospective cohort study. Sex Transm Infect 89:672–678

Looker KJ, Garnett GP (2005) A systematic review of the epidemiology and interaction of herpes simplex virus types 1 and 2. Sex Transm Infect 81:103–107

Looker KJ, Garnett GP, Schmid GP (2008) An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. Bull World Health Organ 86:805–812 A

Louv WC, Austin H, Perlman J, Alexander WJ (1989) Oral contraceptive use and the risk of chlamydial and gonococcal infections. Am J Obstet Gynecol 160:396–402

Martin DH (2012) The microbiota of the vagina and its influence on women's health and disease. Am J Med Sci 343:2–9

Martin H, Richardson B, Nyange P, Lavreys L, Hillier S, Chohan B, Mandaliya K, Ndinya-Achola J, Bwayo J, Kreiss J (1999) Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infect Dis 180:1863–1868

Martin DH, Zozaya M, Lillis RA, Myers L, Nsuami MJ, Ferris MJ (2013) Unique vaginal microbiota that includes an unknown Mycoplasma-like organism is associated with Trichomonas vaginalis infection. J Infect Dis 207:1922–1931

Mauck CK, Callahan MM, Baker J, Arbogast K, Veazey R, Stock R, Pan Z, Morrison CS, Chen-Mok M, Archer DF, Gabelnick HL (1999) The effect of one injection of Depo-Provera on the human vaginal epithelium and cervical ectopy. Contraception 60:15–24

Mcgee ZA, Jensen RL, Clemens CM, Taylor-Robinson D, Johnson AP, Gregg CR (1999) Gonococcal infection of human fallopian tube mucosa in organ culture: relationship of mucosal tissue TNF-alpha concentration to sloughing of ciliated cells. Sex Transm Dis 26:160–165

Mcmillan A, Dell M, Zellar MP, Cribby S, Martz S, Hong E, Fu J, Abbas A, Dang T, Miller W, Reid G (2011) Disruption of urogenital biofilms by lactobacilli. Colloids Surf B: Biointerfaces 86:58–64

Miller WC, Ford CA, Morris M, Handcock MS, Schmitz JL, Hobbs MM, Cohen MS, Harris KM, Udry JR (2004) Prevalence of chlamydial and gonococcal infections among young adults in the United States. JAMA 291:2229–2236

Mirmonsef P, Gilbert D, Zariffard MR, Hamaker BR, Kaur A, Landay AL, Spear GT (2011) The effects of commensal bacteria on innate immune responses in the female genital tract. Am J Reprod Immunol 65:190–195

Morrison CS, Bright P, Wong EL, Kwok C, Yacobson I, Gaydos CA, Tucker HT, Blumenthal PD (2004) Hormonal contraceptive use, cervical ectopy, and the acquisition of cervical infections. Sex Transm Dis 31:561–567

Moscicki AB, Ma Y, Holland C, Vermund SH (2001) Cervical ectopy in adolescent girls with and without human immunodeficiency virus infection. J Infect Dis 183:865–870

Mostad SB, Overbaugh J, Devange DM, Welch MJ, Chohan B, Mandaliya K, Nyange P, Martin HL Jr, Ndinya-Achola J, Bwayo JJ, Kreiss JK (1997) Hormonal contraception, vitamin A

deficiency, and other risk factors for shedding of HIV-1 infected cells from the cervix and vagina. Lancet 350:922–927
Mostad SB, Kreiss JK, Ryncarz AJ, Mandaliya K, Chohan B, Ndinya-Achola J, Bwayo JJ, Corey L (2000) Cervical shedding of herpes simplex virus in human immunodeficiency virus-infected women: effects of hormonal contraception, pregnancy, and vitamin A deficiency. J Infect Dis 181:58–63
Motevaseli E, Shirzad M, Akrami SM, Mousavi A-S, Mirsalehian A, Modarressi MH (2013) Normal and tumour cervical cells respond differently to vaginal lactobacilli, independent of pH and lactate. J Med Microbiol 62:1065–1072
Myer L, Denny L, Telerant R, Souza M, Wright TC Jr, Kuhn L (2005a) Bacterial vaginosis and susceptibility to HIV infection in South African women: a nested case-control study. J Infect Dis 192:1372–1380
Myer L, Kuhn L, Stein ZA, Wright TC Jr, Denny L (2005b) Intravaginal practices, bacterial vaginosis, and women's susceptibility to HIV infection: epidemiological evidence and biological mechanisms. Lancet Infect Dis 5:786–794
Ness RB, Kip KE, Soper DE, Hillier S, Stamm CA, Sweet RL, Rice P, Richter HE (2005) Bacterial vaginosis (BV) and the risk of incident gonococcal or chlamydial genital infection in a predominantly black population. Sex Transm Dis 32:413–417
O'brien JP, Goldenberg DL, Rice PA (1983) Disseminated gonococcal infection: a prospective analysis of 49 patients and a review of pathophysiology and immune mechanisms. Medicine 62:395–406
Ocana VS, Pesce De Ruiz Holgado AA, Nader-Macias ME (1999) Characterization of a bacteriocin-like substance produced by a vaginal Lactobacillus salivarius strain. Appl Environ Microbiol 65:5631–5635
O'hanlon DE, Moench TR, Cone RA (2011) In vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid but not hydrogen peroxide. BMC Infect Dis 11:200
Paavonen T (1994) Hormonal regulation of immune responses. Ann Med 26:255–258
Pabich WL, Fihn SD, Stamm WE (2003) Prevalence and determinants of vaginal flora alterations in postmenopausal women. J Infect Dis 188:1054–1058
Peipert JF, Lapane KL, Allsworth JE, Redding CA, Blume JD, Stein MD (2008) Bacterial vaginosis, race, and sexually transmitted infections: does race modify the association? Sex Transm Dis 35:363–367
Pennock JW, Stegall R, Bell B, Vargas G, Motamedi M, Milligan G, Bourne N (2009) Estradiol improves genital herpes vaccine efficacy in mice. Vaccine 27:5830–5836
Petersen BH, Lee TJ, Snyderman R, Brooks GF (1979) Neisseria meningitidis and Neisseria gonorrhoeae bacteremia associated with C6, C7, or C8 deficiency. Ann Intern Med 90:917–920
Phukan N, Parsamand T, Brooks AE, Nguyen TN, Simoes-Barbosa A (2013) The adherence of Trichomonas vaginalis to host ectocervical cells is influenced by lactobacilli. Sex Transm Infect 89:455–459
Phupong V, Sittisomwong T, Wisawasukmongchol W (2005) Disseminated gonococcal infection during pregnancy. Arch Gynecol Obstet 273:185–186
Poonia B, Wang X, Veazey RS (2006) Distribution of simian immunodeficiency virus target cells in vaginal tissues of normal rhesus macaques: implications for virus transmission. J Reprod Immunol 72:74–84
Prabhala RH, Wira CR (1995) Sex hormone and IL-6 regulation of antigen presentation in the female reproductive tract mucosal tissues. J Immunol 155:5566–5573
Putkonen T, Ebeling K (1950) Gonococci and the menstrual cycle. J Vener Dis Inf 31:263–267
Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, Mcculle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L, Forney LJ (2011) Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A 108(Suppl 1):4680–4687

Ravel J, Brotman RM, Gajer P, Ma B, Nandy M, Fadrosh DW, Sakamoto J, Koenig SS, Fu L, Zhou X, Hickey RJ, Schwebke JR, Forney LJ (2013) Daily temporal dynamics of vaginal microbiota before, during and after episodes of bacterial vaginosis. Microbiome 1:29

Reddy BS, Rastogi S, Das B, Salhan S, Verma S, Mittal A (2004) Cytokine expression pattern in the genital tract of Chlamydia trachomatis positive infertile women – implication for T-cell responses. Clin Exp Immunol 137:552–558

Reid G, Heinemann C, Velraeds M, Van Der Mei HC, Busscher HJ (1999) Biosurfactants produced by Lactobacillus. Methods Enzymol 310:426–433

Rice PA (2005) Gonococcal arthritis (disseminated gonococcal infection). Infect Dis Clin North Am 19:853–861

Robinson K, Cohen T, Colijn C (2012) The dynamics of sexual contact networks: effects on disease spread and control. Theor Popul Biol 81:89–96

Rompalo AM, Hook EW 3rd, Roberts PL, Ramsey PG, Handsfield HH, Holmes KK (1987) The acute arthritis-dermatitis syndrome. The changing importance of Neisseria gonorrhoeae and Neisseria meningitidis. Arch Intern Med 147:281–283

Rosenthal GE, Landefeld CS (1990) The relation of chlamydial infection of the cervix to time elapsed from the onset of menses. J Clin Epidemiol 43:15–20

Røttingen JA, Cameron DW, Garnett GP (2001) A systematic review of the epidemiologic interactions between classic sexually transmitted diseases and HIV: how much really is known? Sex Transm Dis 28:579–597

Ryckman KK, Williams SM, Krohn MA, Simhan HN (2008) Racial differences in cervical cytokine concentrations between pregnant women with and without bacterial vaginosis. J Reprod Immunol 78:166–171

Schiffer JT, Magaret A, Selke S, Corey L, Wald A (2011) Detailed analysis of mucosal herpes simplex virus-2 replication kinetics with and without antiviral therapy. J Antimicrob Chemother 66:2593–2600

Sobel JD (2005) What's new in bacterial vaginosis and trichomoniasis? Infect Dis Clin North Am 19:387–406

Sobel JD, Ferris D, Schwebke J, Nyirjesy P, Wiesenfeld HC, Peipert J, Soper D, Ohmit SE, Hillier SL (2006) Suppressive antibacterial therapy with 0.75 % metronidazole vaginal gel to prevent recurrent bacterial vaginosis. Am J Obstet Gynecol 194:1283–1289

Srinivasan S, Liu C, Mitchell CM, Fiedler TL, Thomas KK, Agnew KJ, Marrazzo JM, Fredricks DN (2010) Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. PLoS One 5:e10197

Stanberry LR, Spruance SL, Cunningham AL, Bernstein DI, Mindel A, Sacks S, Tyring S, Aoki FY, Slaoui M, Denis M, Vandepapeliere P, Dubin G, Glaxosmithkline Herpes Vaccine Efficacy Study Group (2002) Glycoprotein-D-adjuvant vaccine to prevent genital herpes. N Engl J Med 347:1652–1661

Sturm-Ramirez K, Gaye-Diallo A, Eisen G, Mboup S, Kanki PJ (2000) High levels of tumor necrosis factor-alpha and interleukin-1beta in bacterial vaginosis may increase susceptibility to human immunodeficiency virus. J Infect Dis 182:467–473

Svanborg C, Godaly G, Hedlund M (1999) Cytokine responses during mucosal infections: role in disease pathogenesis and host defence. Curr Opin Microbiol 2:99–105

Torsvik V, Ovreas L (2002) Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol 5:240–245

Trent M (2013) Pelvic inflammatory disease. Pediatr Rev/Am Acad Pediatr 34:163–172

Tronstein E, Johnston C, Huang M-L, Selke S, Magaret A, Warren T, Corey L, Wald A (2011) Genital shedding of herpes simplex virus among symptomatic and asymptomatic persons with HSV-2 infection. JAMA 305:1441–1449

Valente AM, Auerswald CL (2013) Gender differences in sexual risk and sexually transmitted infections correlate with gender differences in social networks among San Francisco homeless youth. J Adolesc Health 53:486–491

Vallor AC, Antonio MA, Hawes SE, Hillier SL (2001) Factors associated with acquisition of, or persistent colonization by, vaginal lactobacilli: role of hydrogen peroxide production. J Infect Dis 184:1431–1436

Wang CC, Mcclelland RS, Overbaugh J, Reilly M, Panteleeff DD, Mandaliya K, Chohan B, Lavreys L, Ndinya-Achola J, Kreiss JK (2004) The effect of hormonal contraception on genital tract shedding of HIV-1. AIDS 18:205–209

Wawer MJ, Sewankambo NK, Serwadda D, Quinn TC, Paxton LA, Kiwanuka N, Wabwire-Mangen F, Li C, Lutalo T, Nalugoda F, Gaydos CA, Moulton LH, Meehan MO, Ahmed S, Gray RH (1999) Control of sexually transmitted diseases for AIDS prevention in Uganda: a randomised community trial. Rakai Project Study Group. Lancet 353:525–535

Wira CR, Rossoll RM (1995) Antigen-presenting cells in the female reproductive tract: influence of sex hormones on antigen presentation in the vagina. Immunology 84:505–508

Wira CR, Rossoll RM, Kaushic C (2000) Antigen-presenting cells in the female reproductive tract: influence of estradiol on antigen presentation by vaginal cells. Endocrinology 141:2877–2885

Wise CM, Morris CR, Wasilauskas BL, Salzer WL (1994) Gonococcal arthritis in an era of increasing penicillin resistance. Presentations and outcomes in 41 recent cases (1985–1991). Arch Intern Med 154:2690–2695

Witkin SS, Linhares IM, Giraldo P, Ledger WJ (2007) An altered immunity hypothesis for the development of symptomatic bacterial vaginosis. Clin Infect Dis 44:554–557

Wolner-Hanssen P, Eschenbach DA, Paavonen J, Kiviat N, Stevens CE, Critchlow C, Derouen T, Holmes KK (1990) Decreased risk of symptomatic chlamydial pelvic inflammatory disease associated with oral contraceptive use. JAMA 263:54–59

Workowski K (2013) In the clinic. Chlamydia and gonorrhea. Ann Intern Med 158:ITC2-1

World Health Organization (2009) World health statistics, 2009. WHO Press, Geneva

Xu F, Sternberg MR, Kottiri BJ, Mcquillan GM, Lee FK, Nahmias AJ, Berman SM, Markowitz LE (2006) Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. JAMA 296:964–973

Yudin MH, Landers DV, Meyn L, Hillier SL (2003) Clinical and cervical cytokine response to treatment with oral or vaginal metronidazole for bacterial vaginosis during pregnancy: a randomized trial. Obstet Gynecol 102:527–534

Chapter 12
Sex and Gender Impact Lyme Disease Immunopathology, Diagnosis and Treatment

Alison W. Rebman, Mark J. Soloski, and John N. Aucott

Abstract Lyme disease is an emerging tick-borne disease that is increasingly prevalent across temperate regions of North America and Eurasia. Investigators from the Centers for Disease Control and Prevention (CDC) recently estimated that there are approximately 300,000 cases annually in the United States. Lyme disease presents with several different clinical phenotypes, largely dependent on the stage of the infection and the presence or absence of prior antibiotic treatment. Compared to other infectious and chronic diseases where sex differences are more pronounced, differences in the number of CDC-reported cases of Lyme disease by sex are unremarkable. Although the prevalence of early Lyme disease appears to be relatively equal by sex, late Lyme disease with objective neurologic or rheumatologic findings appears to be more common in males than females. In contrast, subjective syndromes of more tenuous and complex origin such as post-treatment Lyme disease syndrome (PTLDS) and chronic Lyme disease (CLD) appear to be more commonly reported in females than males. Several factors could contribute to these observed differences in clinical presentation between the sexes. While social and behavioral risk factors may play an important role, this chapter will focus on underlying differences in the immune response between males and females following infection, which could affect bacterial clearance, development of autoimmune-like responses, and seroconversion on two-tier antibody tests. In the four decades of research since the discovery of the Lyme spirochete, much remains unknown regarding sex- and gender-based differences in the epidemiology, clinical presentation, and immunologic response to this infection.

A.W. Rebman • J.N. Aucott (✉)
Department of Medicine, Johns Hopkins University, 2360 W. Joppa Rd., Joppa Concourse, Suite 320 Lutherville, MD 21093, USA
e-mail: jaucott@jhmi.edu

M.J. Soloski
Department of Medicine, Johns Hopkins University, Mason F. Lord Building - Center Tower, 5200 Eastern Avenue, Suite 6000, Rm. 677 Baltimore, MD 21224, USA

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_12

12.1 Lyme Disease Overview

Lyme disease, also called Lyme borreliosis, is the most common human tick-borne infection worldwide (Paddock and Telford 2010). This vector-borne disease is transmitted by ticks of the *Ixodes ricinus* complex, the majority of which are found in specific regions of North America, Europe, and Asia (Fig. 12.1). The causative agents of Lyme disease are members of the *Borrelia* genus, which belong to the Spirochaetes phylum of distinctively spiraled or corkscrew-shaped bacteria. Over 30 different genospecies of *Borrelia* have been identified, of which at least five are known to cause human disease in endemic areas. In the Northeast, Upper Midwest, and Pacific Northwest of the United States, *Borrelia burgdorferi sensu stricto* is responsible for most confirmed cases of Lyme disease, whereas in Europe, *Borrelia burgdorferi sensu lato*, *Borrelia garinii*, and *Borrelia afzelii* cause most human disease. Other, novel genospecies of *Borrelia* have recently been identified, some of which carry the possibility for human disease. For example, *Borrelia miyamotoi* was first identified in ticks in 1995, with human infection first described in Russia in 2011 (Platonov et al. 2011) and in the United States in 2013 (Krause et al. 2013).

Although it was only discovered in recent years, Lyme disease is an ancient disease. In 2012, *Borrelia burgdorferi* genetic material was isolated from a bone sample of a 5,300-year-old male preserved in the Italian Alps (Keller et al. 2012). While specific neurologic and dermatologic syndromes due to Lyme disease were recognized in Europe in the early twentieth century, they were not linked to an infectious etiology at the time. In the United States, Lyme disease was first identified in the mid-1970s during a focal outbreak of pediatric arthritis cases in Lyme, Connecticut (Steere et al. 1977). The bacterial etiology was subsequently

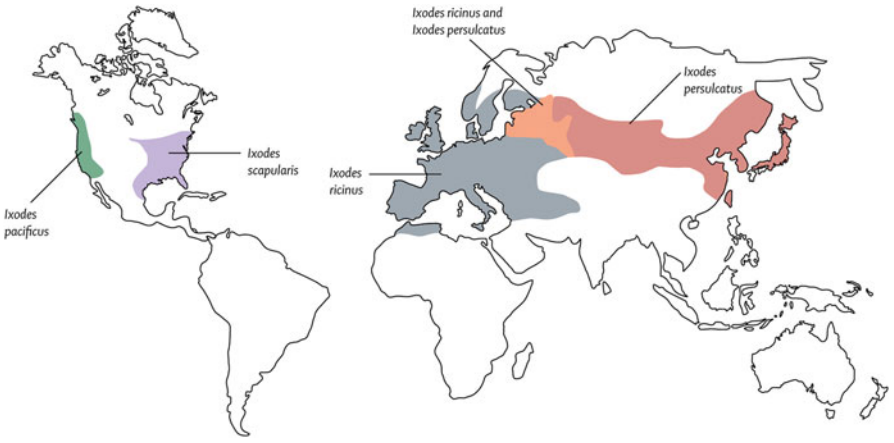

Fig. 12.1 Global distribution of the vectors (*Ixodes ricinus* species complex) of Lyme *Borrelia*. Reprinted from Stanek et al. (2012) with permission from Elsevier

identified and linked conclusively to the tick vector and infected patients in the early 1980s (Burgdorfer et al. 1982).

Over the last four decades, the number of Lyme disease cases has risen sharply, and it is now the most common vector-borne disease in the United States and Europe. This rapid emergence is linked to a complex interaction of human land use patterns with both environmental and species-level biodiversity (Levy 2013). In endemic areas, Lyme disease is often acquired in either peri-urban or rural areas where individuals live or engage in recreational activities. The majority of acute cases of Lyme disease occur between late May and late September, when the infected tick vectors are actively feeding (Fig. 12.2).

Despite nearly four decades of scientific inquiry into the transmission dynamics, immunopathology, and treatment outcomes of Lyme disease, much still remains unknown. Further, there has been a general lack of research examining potential sex- and gender-based differences in this infectious disease setting. This chapter will present a summary of findings, clinical observations, and suggestions for future research priorities across several areas of Lyme disease research including epidemiologic reporting, clinical disease presentation, immunologic response, treatment paradigms, and serological testing.

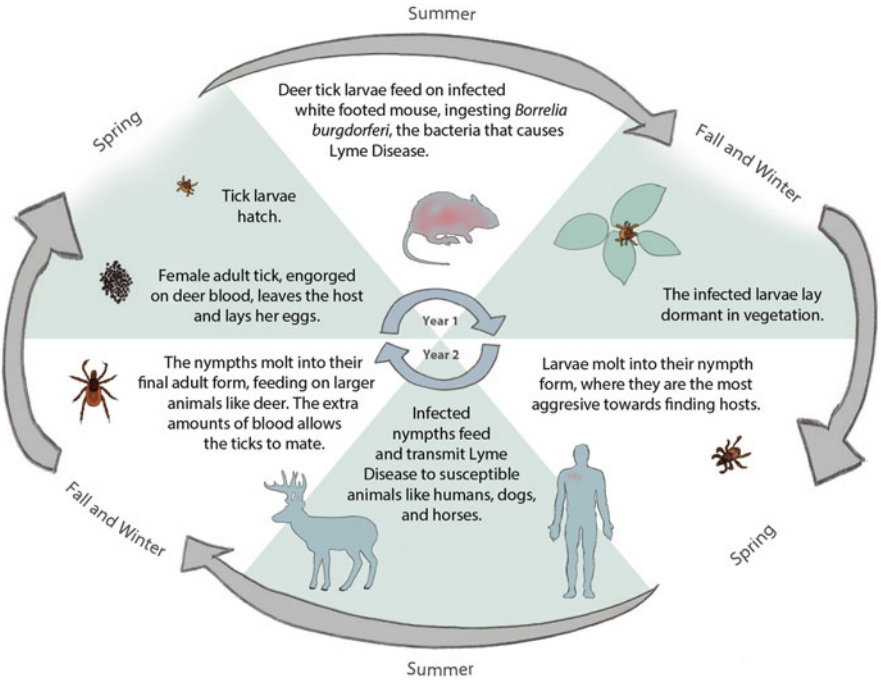

Fig. 12.2 The 2-year life cycle of *Ixodes scapularis* ticks

12.2 Epidemiologic Trends

In the United States, Lyme disease is reportable to the Centers for Disease Control and Prevention (CDC), which recently began classifying cases as either "confirmed" or "probable" (Centers for Disease Control and Prevention 2013a). Over 22,000 confirmed cases were reported in 2012, with an additional 8,000 probable cases reported (Centers for Disease Control and Prevention 2013b). Previous research showed that Lyme disease was commonly underreported through standard surveillance mechanisms (Meek et al. 1996). However, using results from large commercial laboratories, the CDC recently revised this estimate to 300,000 cases yearly in the United States (Kuehn 2013).

Among confirmed cases in the United States, males are a slight (53.1 %) majority (Bacon et al. 2008). This trend may have varied somewhat over time; however, as in previous years (1992–1995), reported cases were more equal by sex (Fig. 12.3). The reason for such shifts are unknown but could include random variation, a change in behavioral risk factors, or changes in state or national level case reporting criteria. These data also appear to show an age effect among reported cases (Fig. 12.4). As described in the 2008 report mentioned above (Bacon et al. 2008), the number of reported cases among young males (age range: 5–19 years) had increased disproportionately compared to young females. Over the age of 60, however, it appears that the number of reported cases evens out among males and females, with a slightly higher number of cases reported among females over age 70.

In contrast to the United States, publications from several areas of Scandinavia and the European continent report that women often represent a slight majority of

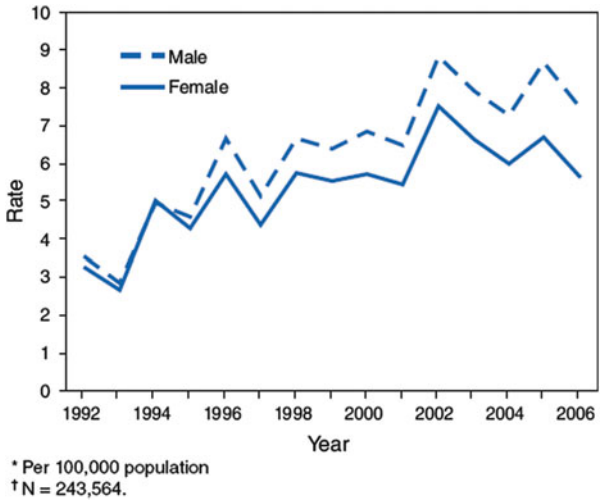

Fig. 12.3 Rate of Lyme disease, by sex and year—United States, 1992–2006 [Reprinted from Bacon et al. (2008)]

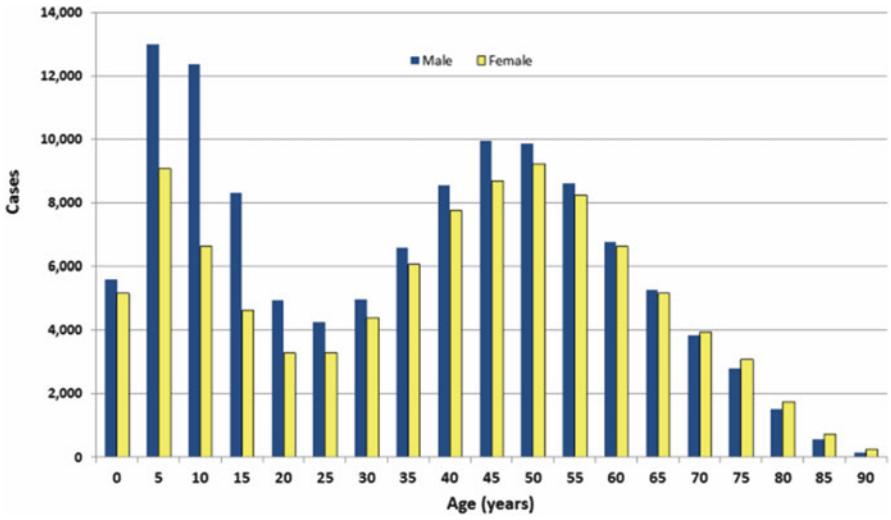

Fig. 12.4 Confirmed Lyme disease cases by age and sex—United States, 2001–2010 [Reprinted from Centers for Disease Control and Prevention (2013c)]

cases of erythema migrans (EM, the diagnostic rash of early Lyme disease) (Mehnert and Krause 2005; Lopes de Carvalho and Núncio 2006; Zöldi et al. 2013). Analysis of surveillance data across six eastern German states found that females were more frequently diagnosed with early Lyme disease (Fülöp and Poggensee 2008). Another study in southern Sweden also found that the annual incidence of EM was significantly higher among women than men (Bennet et al. 2007). The authors found that this difference was most pronounced among women over age 40 and that this demographic group also had the highest risk of attracting tick bites.

National-, regional-, or community-level variation in surveillance methods as well as physician awareness and diagnosis patterns may contribute to epidemiologic trends in case reporting of Lyme disease. The complex ecological drivers that govern prevalence, as well as variability across and within infecting genospecies, likely add additional complexity. For example, Bacon et al. (2008) found that even within the United States, cases of Lyme disease differed by age and sex between the "Healthy People 2010 states" where the disease is endemic and all other states where it is less common. Among endemic states, the modal age was much younger (7 years old) and males accounted for 53 % of cases, whereas in all other states the modal age was 44 years old and males accounted for 49 % of cases.

Compared to other infectious and chronic disease settings where sex differences are pronounced (see Chaps. 5–6 and 13), sex differences in the reported number of cases of confirmed or probable Lyme disease are relatively unremarkable. While behavioral risk factors and the sociological context of exposure and diagnosis are important and warrant further research, this chapter will largely focus on findings concerning sex differences in the underlying biologic response to the infection,

specifically as it may relate to clinical presentation, immunologic response, treatment paradigms, and serological testing.

12.3 Clinical Presentations

In the United States, Lyme disease presents with several different clinical phenotypes which are thought to largely depend on the stage of the infection and the presence or absence of prior antibiotic treatment. Figure 12.5 shows common clinical phenotypes of untreated Lyme disease, the percentage of patients presenting with each phenotype, and the typical number of months from infection to presentation. The presence or absence of antibiotic therapy is important, as the natural history of untreated Lyme disease is very different from that of treated infection, after which a condition called post-treatment Lyme disease syndrome (PTLDS) may occur.

12.3.1 Untreated Borrelia *Infection*

12.3.1.1 Early Lyme Disease

Early Lyme disease is characterized by the EM skin lesion, which is thought to occur in approximately 80 % of cases (Steere 2001). This diagnostic rash is often also accompanied by viral infection-like symptoms such as fever, fatigue, and myalgia. The EM skin lesion can take on a variety of appearances, not all of which correspond to a classic "bull's eye" with concentric rings (Schutzer

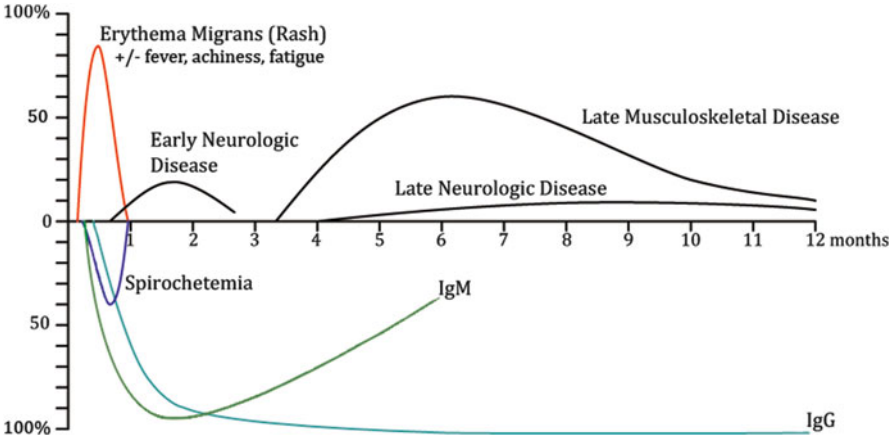

Fig. 12.5 The natural history of untreated Lyme disease

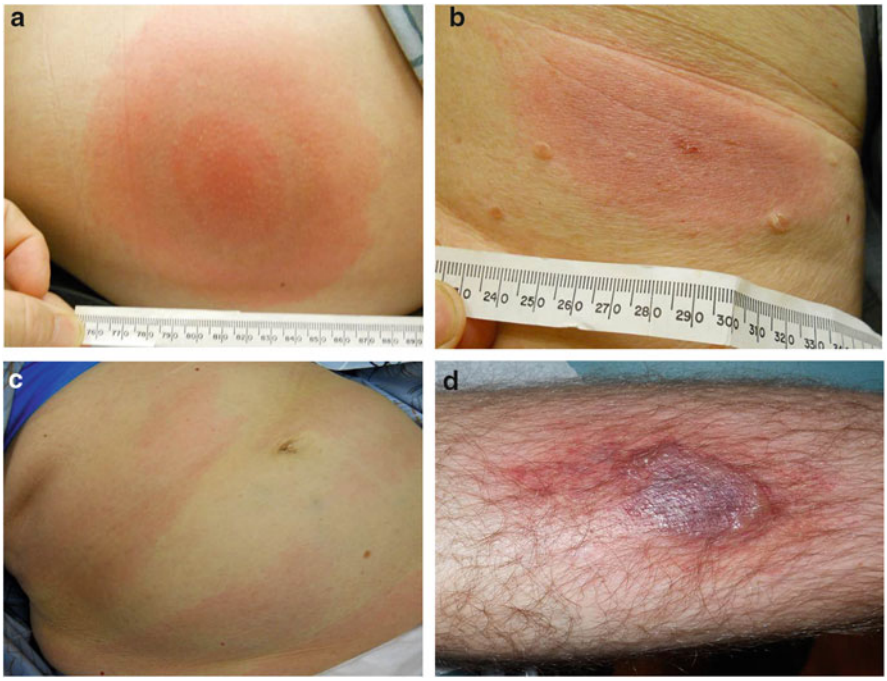

Fig. 12.6 (**a**) A classic, bull's eye target erythema migrans lesion in an IgM-positive early Lyme disease patient. (**b**) A uniformly red erythema migrans lesion in an IgM-positive early Lyme disease patient. (**c**) Disseminated erythema migrans lesions in an IgM- and IgG-positive early Lyme disease patient. (**d**) An atypical erythema migrans lesion with a vesicular central component in an IgM-positive early Lyme disease patient

et al. 2013). Figure 12.6 shows examples of variation in the EM rash, including dissemination of the original rash into multiple lesions, all among patients who had a positive two-tier serological test for Lyme disease. While the diagnosis of most cases of early Lyme disease is based on a physician-confirmed EM, a positive serological test is required to confirm diagnosis of the viral-like presentation of early Lyme disease in cases where the EM is absent or not observed. However, this can be challenging as serological tests are often negative in the first few weeks of infection and have a low sensitivity following early treatment (Aguero-Rosenfeld et al. 1996; Rebman et al. 2014). More detail on the serological tests for Lyme disease is included in Sect. 12.4.

Sex-based differences in the early symptoms of Lyme disease have not been routinely examined or reported. It is not known if there are differences in rash size or appearance between the sexes in the United States; however, one study from Sweden found that women's EM lesions were less likely to have the classic "bull's eye" appearance and took longer to disappear than men's (Bennet et al. 2007). Our data from a large prospective cohort study of early Lyme disease show few differences in the clinical presentation of early Lyme disease by sex. Similar

proportions of males and females were found to have multiple EM lesions, often a marker of dissemination of disease and thus of increased severity of illness. Furthermore, men and women typically self-reported a similar set of symptoms. The exceptions were a significantly higher proportion of females than males reported acute gastrointestinal symptoms, heart palpitations, and at least one elevated liver function test prior to antibiotic treatment as part of their initial clinical presentation (Schwarzwalder et al. 2010b).

Without early treatment, bacteremia may result in signs of dissemination in the weeks or months that follow initial infection. Clinical manifestations of noncutaneous disease can be protean and are usually of neurologic, cardiac, or musculoskeletal origin (Steere 1989). It is not known if one sex is more likely to develop organ involvement during the initial stages of dissemination, although one large European case series of patients with early Lyme central nervous system infection reported a greater percentage of men than women with positive tests for central nervous system involvement (Ogrinc et al. 2013).

12.3.1.2 Late Lyme Disease

Late Lyme disease occurs in cases of untreated early infection and is characterized by delayed manifestations that occur months to years later (Fig. 12.5). The diagnostic criteria for late Lyme arthritis or neurologic disease include physician-documented signs of joint or neurologic pathology and a concurrent positive two-tier serological test. More detail on the serological tests for Lyme disease is included in Sect. 12.4.

In the United States, a significant sex-based difference in patients diagnosed with late Lyme arthritis is observed, with approximately twice as many male as female cases represented in study samples (Steere and Angelis 2006; Kannian et al. 2007). Similarly, our retrospective chart review of patients in a community-based setting also found a majority (61 %) of those presenting with neurologic symptoms or arthritis were male (Aucott et al. 2012). In the European setting, a male predominance for late-stage arthritis and neurologic complications has also been noted in clinic or referral-based patient samples (Renaud et al. 2004; van Burgel et al. 2011). For example, a recent analysis of chart reviews from a university medical center in Slovenia from 1990 to 2012 found that there was a male predominance among patients with Lyme arthritis and among those with neuroborreliosis (Strle et al. 2013). The same study also found a female predominance among patients with acrodermatitis chronica atrophicans, a late dermatologic manifestation that is specific to infection with the *Borrelia afzelii* genospecies found in Europe. The fourfold higher rate of arthritis in men was present in both the overall sample and in the subset of patients with culture or PCR-confirmed arthritis.

The basis for lower diagnosis rates of common objective findings of late Lyme disease among women is unknown. Given approximately equal exposures of both men and women to the early stages of acute infection, it would be expected that rates of later stage clinical phenotypes would also be roughly equivalent. Several

hypotheses could explain this pattern. Women could be less susceptible to more virulent, disseminating strains that cause late objective manifestations and chronic disease. Alternatively, women could have a host response that limits dissemination. Behaviorally, women may be more likely to present to a physician earlier in the infection, resulting in higher rates of prompt early diagnosis and treatment, which would prevent progression to later disease states. Finally, atypical presentations of late Lyme disease may be more common among women, resulting in a decreased ability to diagnose the disease, particularly if such manifestations aren't captured by current case definitions and clinical guidelines.

12.3.2 Post-treatment Lyme Disease Syndrome

PTLDS is a symptom-defined condition that occurs after antibiotic treatment of documented early or late Lyme disease. The Infectious Disease Society of America (IDSA) published a proposed case definition for PTLDS in 2006 (Wormser et al. 2006), but currently no biomarkers have been identified to aid diagnosis or to monitor cure. PTLDS can be defined in patients from Lyme endemic regions with a history of physician-diagnosed and antibiotic-treated Lyme disease. It is characterized by associated symptoms of fatigue, musculoskeletal pain, and cognitive complaints, which result in a measureable decline in health-related function (Aucott et al. 2013).

Results from a variety of study designs show that women are more likely than men to develop PTLDS and subjective symptoms following treatment of Lyme disease. While the initial outcome and registry studies of early Lyme disease found that up to 50 % of patients reported some degree of residual symptoms, the sex of patients in this subset was rarely reported (Steere et al. 1983; Nadelman et al. 1992; Asch et al. 1994; Luft et al. 1996). Two case series of referral patients seen in separate Lyme disease clinics showed a female predominance among those diagnosed with persistent symptoms following treatment (Sigal 1990; Steere et al. 1993). This female bias becomes more striking in studies such as Sigal's (1990) that specifically included a fibromyalgia syndrome in their definition of PTLDS. In this instance, the female/male ratio was approximately 8:1 (Sigal 1990). In contrast, a large population-based study found that female sex was not associated with PTLDS (Shadick et al. 1994).

Few prospective, controlled studies have been performed to look at the impact of patient sex on the risk or severity of PTLDS. Data from our prospective study of patients with early Lyme disease show that women were more likely than men to develop new subjective symptoms and meet a rigorous case definition for PTLDS following treatment of Lyme disease (unpublished data). European studies of Lyme disease with long-term follow-up of patients after treatment for neuroborreliosis show that female sex may be a risk factor for persistent symptoms. One study identified an odds ratio of 3:2 for women developing post-treatment symptoms 1 year after antibiotic treatment (Ljøstad and Mygland 2010). However, in another

study, sex was not significant in predicting health-related quality of life or fatigue after treatment of neuroborreliosis (Eikeland et al. 2013). Many of the above studies suggest that women may be at a higher risk of developing PTLDS, especially when fibromyalgia syndrome is considered as a broader subset of PTLDS phenotype.

Several hypotheses could explain why women may be overrepresented in the PTLDS group following treatment. As previously mentioned, if women seek treatment earlier in the course of Lyme disease, development of late objective disease may largely be prevented. At the same time, however, earlier treatment may place more women at risk for subjective, post-treatment sequelae. Recent research on PTLDS has shifted from behavioral risk factors and characterization of the disease toward potential immune-mediated mechanisms of the largely subjective symptoms that characterize the syndrome. More detail on the immunologic context is described in Sect. 12.6.

12.3.3 Chronic Lyme Disease

In the medical literature, chronic Lyme disease (CLD) is often considered a nonspecific diagnosis which is distinct from both untreated infection and PTLDS (Feder et al. 2007). While CLD and PTLDS share many similarities in the subjective symptoms of fatigue, pain, neurocognitive dysfunction, and others, patients often seek care for or are given the diagnostic label of CLD when current or past evidence of *Borrelia* infection cannot be determined. A review of antibiotic treatment studies for CLD found female to male ratios which ranged from 1.8 to 2.8 (Wormser and Shapiro 2009). Similarly, in our retrospective chart review of patients, we found that women represented 66 % of patients with medically unexplained symptoms that could not be given a diagnosis using current CDC and IDSA guidelines for early Lyme disease, late Lyme disease, or PTLDS (Aucott et al. 2012).

It has been noted that this group of patients almost certainly represents a heterogeneous subset, likely including those with PTLDS as well as cases with medically unexplained symptoms. Several publications have asserted that the female predominance in CLD is a result of misdiagnosed fibromyalgia, chronic fatigue syndrome, or depression, all conditions with similarly high female to male ratios (Sigal 1990; Hsu et al. 1993; Wormser and Shapiro 2009). There is considerable symptom overlap between CLD, PTLDS, and these other symptom-based syndromes, and it remains difficult if not impossible to separate them clinically. Complicating diagnosis further is the observation that Lyme disease is one of the recognized infectious triggers of fibromyalgia, which likely represents an end-stage phenotype of many different illness processes (Dinerman and Steere 1992; Sigal and Patella 1992; Hsu et al. 1993).

Other authors have suggested that CLD symptoms are often instead attributable to psychiatric disorders or other underlying psychological issues. For example, in a sample of largely female (83 %) CLD patients, higher rates of psychiatric disorders

and a greater tendency toward catastrophizing were found when compared to those with PTLDS, those who had recovered from Lyme disease, and those with an alternate diagnosis (Hassett et al. 2008; Hassett and Radvanski 2009). However, as acknowledged by the authors, these cross-sectional studies cannot account for the effects of a medically unexplained, often long-standing illness on mental health status.

It should also be noted that due to potential misdiagnosis in community settings (Aucott et al. 2009), it is unknown what percentage of these patients may have the sequelae of remote, unrecognized tick-borne disease (Aucott et al. 2012). Indeed, patients who carry the diagnosis of CLD are often found to have little or no serological evidence for prior infection. While this observation is often used as additional evidence for lack of prior exposure to *B. burgdorferi*, the limitations of currently available antibody tests in antibiotic exposed patients (described in more detail in Sect. 12.4) (Aguero-Rosenfeld et al. 1996; Rebman et al. 2014) may argue for a more detailed assessment of such patients. Above all, these observations highlight the heterogeneity of these populations and the difficulty of identifying etiology without a clinically available biomarker test of either direct infection or cure.

12.3.4 Similarities to Other Bacterial Infections

Sex-based differences in clinical presentation have been documented in other bacterial disease settings, including those mentioned in Chap. 11. Perhaps the most relevant is that of syphilis, another spirochetal infection. *Borrelia burgdorferi* shares both microbiological resemblance and clinical symptom similarities with its spirochete cousin, *Treponema pallidum*, the infectious agent of syphilis.

Before the advent of penicillin in the 1940s, Joseph Earle Moore performed several early studies at the Syphilis Clinic of the Johns Hopkins Hospital and documented that while early neurosyphilis was equally common among men and women, clinical neurosyphilis was three to four times as likely to occur among men, adding to an even earlier literature and body of statistics showing the same trend (Moore 1922a). While he found this discrepancy compelling, he found it even more surprising that cerebrospinal fluid abnormalities, and thus nervous system invasion, were equally common among men and women who had been infected. He speculated that pregnancy, as the "most important point of difference in the ordinary life history of the two sexes" (Moore 1922b), may play a mediating role in exempting women from frank neurosyphilis despite cerebrospinal fluid abnormalities. Indeed, his studies among patients in the same clinic showed that pregnancy was protective in lowering the risk of neurosyphilis as well as grave syphilitic lesions. A lower rate of these complications was found both in cases of infection acquired at the time of conception, as well as among women who became pregnant during the early stage of the disease (Moore 1922b). The protective effects of female sex and pregnancy on development of objective neurologic findings in syphilis may be relevant to

explore further in the context of Lyme disease and may inform hypothesis generation regarding hormonal effects on the inflammatory immune response.

12.4 Laboratory Testing

The current laboratory testing guidelines for clinical diagnosis of Lyme disease rely on a two-tier strategy for antibody testing. An ELISA (enzyme-linked immunosorbent assay) measuring total anti-borrelia IgM and IgG antibodies is the first tier of testing. If the resulting value is above previously determined cutoffs, the ELISA screening test is considered positive, and a confirmatory Western blot test is performed during the second tier of testing. The Western blot test detects the presence of specific IgM (immunoglobulin M) and IgG (immunoglobulin G) anti-borrelia antibodies (Centers for Disease Control and Prevention 1995).

CDC guidelines recommend that interpretation of the results of these tests (ELISA and reflex Western blot) is guided by the duration of illness reported by the patient at the time of evaluation (Centers for Disease Control and Prevention 2011). Those patients with signs and symptoms of less than thirty days are considered to have a positive test in the context of a positive ELISA and either a positive IgM or IgG Western blot. However, in patients with greater than 30 days' illness duration, a positive ELISA and a positive IgG Western blot is required for serological confirmation of exposure to *B. burgdorferi* (Centers for Disease Control and Prevention 2011). This two-tier strategy, an approach implemented to capture a high degree of specificity for case surveillance, is limited by a low sensitivity in the diagnosis of early Lyme disease and in convalescent testing after early treatment (Aguero-Rosenfeld et al. 1996; Rebman et al. 2014).

A handful of studies have examined differences in sex-based serological reactivity on commercially available tests in the diagnosis of Lyme disease. In our retrospective chart review of patients with early Lyme disease in a community setting (Schwarzwalder et al. 2010a), we found that women had a significantly lower ELISA value and a significantly lower total number of reactive IgG Western blot bands than men. Figure 12.7 shows enhanced box plots of the difference in results by sex for these two tests. We found a similar trend using data from a prospective cohort of patients with early Lyme disease diagnosed by physician-confirmed EM. These results also suggested that men were more likely than women to end up with a positive test from the entire clinical encounter when tested and treated according to current guidelines (Rebman et al. 2014). Notably, both study designs in separate sample populations found that while similar proportions of males and females were positive during acute infection prior to antibiotic exposure, men were more likely to have a positive Western blot (typically IgG) test on a convalescent, post-treatment serology. Similarly, in one antibiotic treatment trial among patients with PTLDS, women were found to be more commonly negative on serological evaluation for *Borrelia* antibodies than men (Klempner et al. 2001). Not all studies have agreed with these findings. A cross-sectional study of acute patients

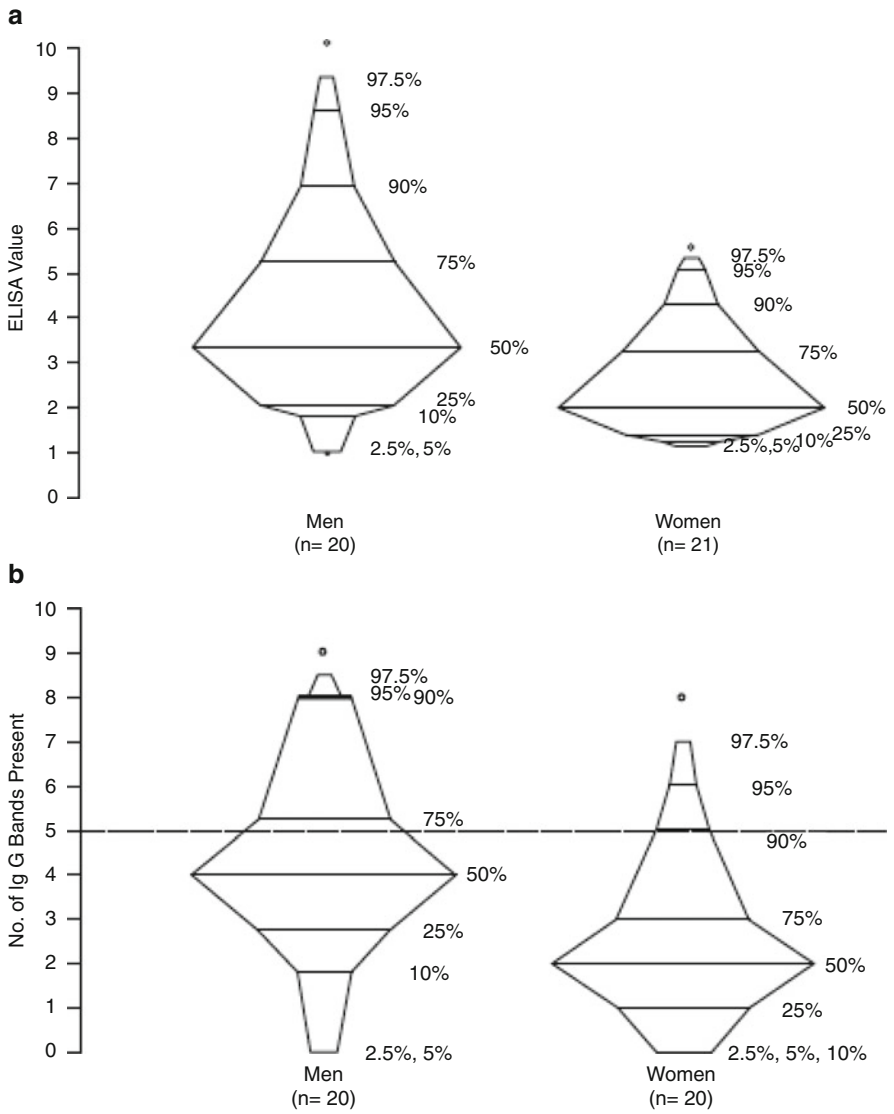

Fig. 12.7 (**a**) Distribution of ELISA results among men and women with a positive or equivocal value (>0.90) by sex. Median values were 3.4 for men and 2.0 for women ($p = 0.03$) [Reprinted from Schwarzwalder et al. (2010a) with permission from Elsevier]. (**b**) Distribution of number of immunoglobulin G (IgG) bands present among men and women with a positive or equivocal ELISA result (>0.90) by sex. Median number of bands were four for men and two for women ($p = 0.03$) [Reprinted from Schwarzwalder et al. (2010a) with permission from Elsevier]

with early Lyme disease at a single time point showed that there were no differences on ELISA value alone nor on the standard two-tier serology in the first 4 weeks of disease (Wormser et al. 2008).

Although there is little precedent for sex-based differences in serological antibody responses against *Borrelia* spp., it has been previously documented in other infectious settings. In two hepatitis A outbreaks in Crete, women were found to have a significantly higher cumulative probability of IgM anti-HAV antibodies (Hatzakis and Hadziyannis 1984). Furthermore, elevated humoral immune responses, cell-mediated immunity, and adverse reactions in females compared with males have been documented in a range of vaccine studies (Green et al. 1994; Klein et al. 2010).

12.5 Treatment Paradigms

There is significant evidence that sex-based differences in the pharmacokinetics and pharmacodynamics of drugs may occur as a result of a range of biologic factors (Gandhi et al. 2004; Nicolas et al. 2009; Franconi et al. 2012). Further, it has been noted that the same plasma concentration of a drug in males and females does not necessarily result in the same pharmacological outcome (Fish 2008). Antibiotic treatment recommendations for previously untreated early and late Lyme disease are not currently different for men and women. According to the most recent IDSA guidelines (Wormser et al. 2006), oral therapy with either doxycycline, amoxicillin, or cefuroxime axetil is recommended for most cases. In cases with early neurologic disease, cardiac disease, or late cases of recurrent arthritis, parenteral therapy with ceftriaxone is recommended instead (Wormser et al. 2006).

To our knowledge, the pharmacokinetics of the recommended therapies listed above have not been evaluated for sex-based differences in the context of *Borrelia* infection, and it is not known how this may affect bacterial clearance, risk of relapse, or development of post-treatment symptoms. In one study among healthy volunteers in Vietnam, women had a statistically longer time to maximum drug concentration of doxycycline; however, all other pharmacokinetic markers were not significantly different (Binh et al. 2009). This is similar to results of a study among a small sample of geriatric patients (Saivin and Houin 1988), but different from an earlier study which found higher peak plasma concentrations of doxycycline among women, even after controlling for body weight (CollaGenex Pharmaceuticals 1998). Studies among small samples of healthy individuals have not found statistically significant sex-based differences in plasma concentration-time curves for cefuroxime axetil (Williams and Harding 1984) or ceftriaxone (Pletz et al. 2004). We are not aware of any studies on the pharmacokinetics of amoxicillin which have specifically focused on sex-based differences.

There are currently no FDA-approved treatments for PTLDS, as the underlying pathophysiology of the symptoms remains unclear. The IDSA guidelines currently do not recommend additional antibiotic therapy for cases where a recommended course of antibiotics has already been given or for cases of CLD in which an established link to past or current *Borrelia* infection is more tenuous (Wormser et al. 2006). Previous controlled trials have failed to provide convincing evidence

that additional antibiotics provide sustained improvements (Klempner et al. 2001; Krupp et al. 2003; Fallon et al. 2008); however, these results still elicit current methodological contestation in the literature (Klempner et al. 2013; Fallon et al. 2014). Further, more recent findings have suggested a potential role for antibiotic-tolerant persistent organisms in vitro and in animal models (Hodzic et al. 2008; Embers et al. 2012; Feng et al. 2014), which may challenge existing treatment paradigms and argue for the development of novel antimicrobial treatment approaches.

In lieu of a proven biological approach to cure, symptom-based or supportive management of pain, fatigue, or other subjective complaints is sometimes recommended for PTLDS or CLD (Wormser et al. 2006). Similarly, psychological or psychosocial modalities such as cognitive behavior therapy or other interventions to strengthen mood, coping, and life functioning have been advocated for these patients (Hassett et al. 2008). It has also been suggested that drug therapies such as antidepressants or agents approved for fibromyalgia such as pregabalin may alleviate symptoms (Lantos 2011). Although not recommended by current IDSA guidelines, complementary and alternative medicine modalities are often utilized by patients with PTLDS and CLD (Ali et al. 2014). All of these remain untested however, as no studies have been performed to examine their effect and quantify potential benefit to this patient population. Future research is needed which can appropriately account for sex-based differences in outcome status as well as assess the efficacy of all possible treatment approaches and consider how they may differentially benefit men and women.

12.6 Immune Response

There is a large body of data from both animal models and in the human setting showing that sex-specific factors clearly influence the host immune response. Despite this, studies on the role of sex-based differences in the immune response to *B. burgdorferi* are lacking. A broad search of PubMed using the terms gender/sex, immunity, and Lyme revealed a total of five papers, two of which were relevant. This represents a serious knowledge gap since region-specific sex-based disparities have been reported in Lyme disease clinical manifestations (Bacon et al. 2008; Schwarzwalder et al. 2010a; Strle et al. 2013). Importantly, PTLDS, one of the confounding downstream outcomes of Lyme disease in which immune-mediated processes are hypothesized to have a role, is clearly female biased (Wormser and Shapiro 2009). Moreover, it has been observed with a related *Borrelia* species (*B. hermsii*) that female mice control bacteria levels more effectively than males (Benoit et al. 2010). This section will present an overview of our current knowledge on the host immune response to *B. burgdorferi*, and when applicable, sex-specific information will be highlighted.

Infection with *B. burgdorferi* leads to the early activation of a number of components of the innate immune system, as demonstrated in murine models as

well as in humans. For example, in the EM skin lesion during acute human Lyme disease, monocytoid and CD11c- plasmacytoid dendritic cell (DC) activation is seen, and blood plasmacytoid DCs display increases in CD80 expression (Salazar et al. 2003). The activation and maturation of DCs following *B. burgdorferi* exposure is mediated by toll-like receptor (TLR) signaling, as *B. burgdorferi* lipoproteins are ligands for TLR1/2 heterodimers (Alexopoulou et al. 2002; Hirschfeld et al. 1999). TLR7- and TLR9-mediated recognition of *B. burgdorferi* drives a type I interferon response in plasmacytoid DCs and CD14+CD11c+ cells (Petzke et al. 2009). The role of TLR1/2 is supported by mouse studies where deficiencies in MyD88, TLR1, or TLR2 impair clearance of *B. burgdorferi* but the development of downstream inflammatory events such as arthritis are unaffected (Alexopoulou et al. 2002; Liu et al. 2004; Bolz et al. 2004). These studies did not consider sex-specific influences. This may be an important issue since in humans and mice, the TLR7 gene is located on the X chromosome, and sex differences in TLR2 expression and signaling contribute toward survival from Coxsackie B infection (Roberts et al. 2012, 2013).

Natural killer T cells (NKT), a thymus-derived T cell subset that also displays innate-like functions, have been found to recognize *B. burgdorferi* glycolipid II presented by the CD1d molecule (Kinjo et al. 2006; Bendelac et al. 2007). Mice lacking NKT cells display diminished clearance of *B. burgdorferi* and increased joint disease following tick-borne infection, indicating a critical role for this subset (Kinjo et al. 2006; Tupin et al. 2008). Differences by sex were not addressed in these studies, and this may also be a relevant area of study since sex-based differences in NKT cell levels have been reported in normal adults (Kee et al. 2012).

The humoral immune response plays a clear role in clearance of *B. burgdorferi* (McKisic et al. 2000; McKisic and Barthold 2000; LaRocca and Benach 2008). This is supported by the extensive pathology seen in B cell-deficient mice and the ability of antibody or B cells to passively protect immune-deficient mice (Schaible et al. 1990; McKisic and Barthold 2000; Barthold et al. 2006). In addition, T cell-independent and T cell-dependent antibody responses have been described, indicating the involvement of B1 and B2 subsets of B cells (McKisic and Barthold 2000). Interestingly, in the above antibody transfer studies, female mice were utilized but no comparison was made with males (Schaible et al. 1990).

Animal models have also demonstrated a critical role for $CD4^+$ T cells. CD4 deficient female mice were highly susceptible to infection with *B. burgdorferi* (Keane-Myers and Nickell 1995). In studies using female mice, the polarization of $CD4^+$ Th1 vs. Th2 subsets was shown to influence the severity of arthritis and susceptibility to infection (Matyniak and Reiner 1995; Kang et al. 1997). In humans, $CD4^+$ Th1 and Th17 cells have been found in Lyme arthritis joints (Yin et al. 1997; Gross et al. 1998; Codolo et al. 2008, 2013). CD8+ T cells are also activated following infection in mouse models as well as in EM lesions, but their precise role is poorly understood (Busch et al. 1996; Dong et al. 1997).

The balance between Th1 (effector) versus Th2 (helper) CD4 T cell effector function impacts the clinical outcome in many disease settings. In studies

identifying Th1 and TH17 CD4 T cells in human Lyme arthritis, both males and females were included in the small study groups but no overt differences were noted (Yin et al. 1997; Gross et al. 1998; Codolo et al. 2008, 2013). In a study of Lyme disease in Sweden, postmenopausal women with a history of Lyme disease were reported to have Th2-directed immune responses with increased cytokine ratios of IL-4/IFN-γ and IL-10/TNF-α compared to men (Jarefors et al. 2006). The authors hypothesized that an increased Th2 response is related to increased risk of reinfection with *Borrelia*. The tendency toward a Th2 response in early infection may also be related to the observation, also in a Swedish cohort, that women were found to have slower resolution of their EM lesions compared with men (Bennet et al. 2007). The elevated Th2 response would predict that females display a heightened antibody response. Surprisingly, in the study of a smaller cohort in the United States, the opposite is observed with women displaying a lower magnitude antibody response than men (Schwarzwalder et al. 2010a). There are numerous possibilities that can account for this seemingly disparate outcome including differences in study group, size of the cohorts, tick species involved in transmission, and the *Borrelia* species that causes Lyme disease. Clearly, this speaks to the need for further study in multiple large cohorts.

Recently the levels of immune mediators in serum were measured in patients with acute Lyme disease (Strle et al. 2012; Soloski et al. 2014). These studies identified two groups of patients, one displaying high levels of serum mediators (chemokines, inflammatory cytokines) and a second with low levels. The elevated mediators were typical of a Th1-type inflammatory response. In one study, comparison of the mediator high vs. mediator low groups did not reveal a sex-based difference (Soloski et al. 2014). In the second study, a sex-based analysis was not reported but an association of high mediator levels was associated with a polymorphism in the TLR1 gene (Strle et al. 2012). It is interesting to point out that females have stronger proinflammatory (Th1) responses than males (reviewed in Klein 2012). Of note, the studies that led to this conclusion were largely generated investigating responses to viruses or viral vaccines so the lack of a sex-based differences in early Lyme may reflect the bacterial etiology of Lyme disease. Alternatively, sex-based difference in the host immune response initiated in Lyme disease may be revealed with more in-depth analysis of the innate and adaptive cellular elements that are mobilized during early and late manifestations of Lyme disease.

It is well documented that many autoimmune diseases show a female bias. There are several lines of evidence that autoimmune processes are a component of Lyme disease. A subset of patients that develop Lyme arthritis are antibiotic refractory, implying an ongoing immune-mediated inflammatory process (Steere 2001). Antibodies toward endothelial cell growth factor (ECGF) are a common feature of Lyme disease, and ECGF is elevated in the synovial fluid of patients with antibiotic-refractory arthritis (Drouin et al. 2013). Interestingly, elevated levels of anti-ECGF and the Th17 associated cytokine IL-23 were associated with PTLDS in a European patient cohort and have been hypothesized to contribute to the development of PTLDS (Strle et al. 2014). Consistent with previous studies, patients with PTLDS

from this cohort were biased 2:1 female/male, but it should be noted that the initial Lyme cohort utilized in this study had a female/male ratio of 1.77:1. Nevertheless, Th17 CD4 cells have been associated with tissue-mediated injury and are implicated in a range of human autoimmune diseases (Peters et al. 2011). It is interesting to point out that dysregulation of Th17 cells has been observed in systemic lupus erythematosus, an autoimmune disease with a strong female bias (Shah et al. 2010). Collectively, these studies indicate that autoimmune-mediated tissue injury may contribute to diverse disease outcomes that follow infection with *B. burgdorferi*.

12.7 Conclusions

Research initiatives examining potential sex- and gender-based differences, as well as the clinical implications of these findings, have been growing across many diverse disease settings, from chronic diseases such as heart disease to infectious diseases such as HIV (see Chap. 5) and tuberculosis (see Chap. 8). In many cases, it has been demonstrated that such differences not only exist, but provide important insights across levels of analysis from behavioral risk factors to the intricacies of the immune response. Lyme disease, with a growing number of cases and significant clinical and geographic variation, is a notably complex infection of relatively recent identification, and much still remains unknown.

This chapter has focused primarily on sex-based findings and observations which relate to the clinical encounter: recognized disease presentations, laboratory testing on commercially available tests, and treatment paradigms. Although early untreated Lyme disease appears to be relatively equal by sex, late untreated Lyme disease with objective neurologic or rheumatologic findings appears to be more frequently found in males, whereas subjective syndromes of more tenuous and complex origin such as PTLDS and CLD appear to be more frequently found in females. Larger, population-based studies will be needed to directly address these observations, as underlying biologic or sociologic explanations have not been identified.

This chapter has also focused on sex-based differences in the innate and humoral immune response to infection with *Borrelia*. This is of particular importance as future research will also be needed to address current limitations in the antibody tests for Lyme disease, as well as pursue development of sensitive biomarkers to distinguish and differentiate all clinical stages of infection and treatment success. Central to the sensitivity and specificity of novel biomarkers will be the performance of such tests in both male and female patient populations. Until biomarkers are developed to identify patients with PTLDS, the percentage of patients with Lyme disease-triggered fibromyalgia, chronic fatigue syndrome, or depression will remain unknown. While the lack of research specifically addressing sex- and gender-based differences in the field of Lyme disease leaves many questions unanswered, it also offers an opportunity to gain rich insight into overall disease pathogenesis and effective treatments for patients across the clinical spectrum.

References

Aguero-Rosenfeld ME, Nowakowski J, Bittker S et al (1996) Evolution of the serologic response to Borrelia burgdorferi in treated patients with culture-confirmed erythema migrans. J Clin Microbiol 34:1–9

Alexopoulou L, Thomas V, Schnare M et al (2002) Hyporesponsiveness to vaccination with Borrelia burgdorferi OspA in humans and in TLR1- and TLR2-deficient mice. Nat Med 8:878–884. doi:10.1038/nm732

Ali A, Vitulano L, Lee R et al (2014) Experiences of patients identifying with chronic Lyme disease in the healthcare system: a qualitative study. BMC Fam Pract 15:79. doi:10.1186/1471-2296-15-79

Asch ES, Bujak DI, Weiss M et al (1994) Lyme disease: an infectious and postinfectious syndrome. J Rheumatol 21:454–461

Aucott JN, Morrison C, Munoz B et al (2009) Diagnostic challenges of early Lyme disease: lessons from a community case series. BMC Infect Dis 9:79

Aucott J, Seifter A, Rebman A (2012) Probable late lyme disease: a variant manifestation of untreated Borrelia burgdorferi infection. BMC Infect Dis 12:173. doi:10.1186/1471-2334-12-173

Aucott JN, Rebman AW, Crowder LA, Kortte KB (2013) Post-treatment Lyme disease syndrome symptomatology and the impact on life functioning: is there something here? Qual Life Res 22:75–84. doi:10.1007/s11136-012-0126-6

Bacon R, Kugeler K, Mead P (2008) Surveillance for Lyme disease – United States, 1992–2006. Morb Mortal Wkly Rep 57:1–9

Barthold SW, Hodzic E, Tunev S, Feng S (2006) Antibody-mediated disease remission in the mouse model of Lyme borreliosis. Infect Immun 74:4817–4825. doi:10.1128/IAI. 00469-06

Bendelac A, Savage PB, Teyton L (2007) The biology of NKT cells. Annu Rev Immunol 25:297–336. doi:10.1146/annurev.immunol.25.022106.141711

Bennet L, Stjernberg L, Berglund J (2007) Effect of gender on clinical and epidemiologic features of Lyme borreliosis. Vector Borne Zoonotic Dis 7:34–41

Benoit VM, Petrich A, Alugupalli KR et al (2010) Genetic control of the innate immune response to Borrelia hermsii influences the course of relapsing fever in inbred strains of mice. Infect Immun 78:586–594. doi:10.1128/IAI.01216-09

Binh VQ, Chinh NT, Thanh NX et al (2009) Sex affects the steady-state pharmacokinetics of primaquine but not doxycycline in healthy subjects. Am J Trop Med Hyg 81:747–753. doi:10.4269/ajtmh. 2009.09-0214

Bolz DD, Sundsbak RS, Ma Y et al (2004) MyD88 plays a unique role in host defense but not arthritis development in Lyme disease. J Immunol 173:2003–2010

Burgdorfer W, Barbour AG, Hayes SF et al (1982) Lyme disease-a tick-borne spirochetosis? Science 216:1317–1319

Busch DH, Jassoy C, Brinckmann U et al (1996) Detection of Borrelia burgdorferi-specific CD8+ cytotoxic T cells in patients with Lyme arthritis. J Immunol 157:3534–3541

Centers for Disease Control and Prevention (1995) CDC. Recommendations for test performance and interpretation from the second national conference on serologic diagnosis of Lyme disease. Morb Mortal Wkly Rep 44:590–591

Centers for Disease Control and Prevention (2011) Two-step laboratory testing process. Lyme Dis. http://www.cdc.gov/mmwr/preview/mmwrhtml/00038469.htm

Centers for Disease Control and Prevention (2013a) Reported cases of Lyme disease by state or locality, 2002–2011. Lyme Dis. http://www.cdc.gov/lyme/stats/chartstables/reportedcases_statelocality.html. Accessed 26 Jun 2013

Centers for Disease Control and Prevention (2013b) Notice to readers: final 2012 reports of nationally notifiable infectious diseases. Morb Mortal Wkly Rep 62:669–682

Centers for Disease Control and Prevention (2013c) Confirmed Lyme disease cases by age and sex – United States, 2001–2010. Lyme Dis. http://www.cdc.gov/lyme/stats/chartstables/incidencebyagesex.html. Accessed 17 Feb 2014

Codolo G, Amedei A, Steere AC et al (2008) Borrelia burgdorferi NapA-driven Th17 cell inflammation in Lyme arthritis. Arthritis Rheum 58:3609–3617. doi:10.1002/art.23972

Codolo G, Bossi F, Durigutto P et al (2013) Orchestration of inflammation and adaptive immunity in Borrelia burgdorferi-induced arthritis by neutrophil-activating protein A. Arthritis Rheum 65:1232–1242. doi:10.1002/art.37875

Collagenex Pharmaceuticals (1998) Periostat Drug Label. http://www.accessdata.fda.gov/drugsatfda_docs/label/1998/50744lbl.pdf. Accessed 18 Sep 2014

Dinerman H, Steere AC (1992) Lyme disease associated with fibromyalgia. Ann Intern Med 117:281–285

Dong Z, Edelstein MD, Glickstein LJ (1997) CD8+ T cells are activated during the early Th1 and Th2 immune responses in a murine Lyme disease model. Infect Immun 65:5334–5337

Drouin EE, Seward RJ, Strle K et al (2013) A novel human autoantigen, endothelial cell growth factor, is a target of T and B cell responses in patients with Lyme disease. Arthritis Rheum 65:186–196. doi:10.1002/art.37732

Eikeland R, Mygland Å, Herlofson K, Ljøstad U (2013) Risk factors for a non-favorable outcome after treated European neuroborreliosis. Acta Neurol Scand 127:154–160. doi:10.1111/j.1600-0404.2012.01690.x

Embers ME, Barthold SW, Borda JT et al (2012) Persistence of Borrelia burgdorferi in rhesus macaques following antibiotic treatment of disseminated infection. PLoS One 7:e29914. doi:10.1371/journal.pone.0029914

Fallon BA, Keilp JG, Corbera KM et al (2008) A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy. Neurology 70:992–1003. doi:10.1212/01.WNL.0000284604.61160.2d

Fallon BA, Petkova E, Keilp JG, Britton CB (2014) Ongoing discussion about the US clinical Lyme trials. Am J Med 127:e7. doi:10.1016/j.amjmed.2013.09.005

Feder HM, Johnson BJB, O'Connell S et al (2007) A critical appraisal of "chronic Lyme disease". N Engl J Med 357:1422–1430. doi:10.1056/NEJMra072023

Feng J, Wang T, Shi W et al (2014) Identification of novel activity against Borrelia burgdorferi persisters using an FDA approved drug library. Emerg Microbes Infect 3:e49. doi:10.1038/emi.2014.53

Fish EN (2008) The X-files in immunity: sex-based differences predispose immune responses. Nat Rev Immunol 8:737–744. doi:10.1038/nri2394

Franconi F, Sanna M, Straface E et al (2012) Pharmacokinetics and pharmacodynamics: the role of sex and gender. In: Oertelt-Prigione S, Regitz-Zagrosek V (eds) Sex and gender aspects in clinical medicine (SE – 12). Springer, London, pp 183–194

Fülöp B, Poggensee G (2008) Epidemiological situation of Lyme borreliosis in Germany: surveillance data from six Eastern German States, 2002 to 2006. Parasitol Res 103(Suppl 1): S117–S120. doi:10.1007/s00436-008-1060-y

Gandhi M, Aweeka F, Greenblatt RM, Blaschke TF (2004) Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol 44:499–523. doi:10.1146/annurev.pharmtox.44.101802.121453

Green MS, Shohat T, Lerman Y et al (1994) Sex differences in the humoral antibody response to live measles vaccine in young adults. Int J Epidemiol 23:1078–1081

Gross DM, Steere AC, Huber BT (1998) T helper 1 response is dominant and localized to the synovial fluid in patients with Lyme arthritis. J Immunol 160:1022–1028

Hassett AL, Radvanski DC (2009) Psychiatric comorbidity and other psychological factors in patients with "chronic Lyme disease.". Am J Med 122:843–850. doi:10.1016/j.amjmed.2009.02.022.Psychiatric

Hassett AL, Radvanski DC, Buyske S et al (2008) Role of psychiatric comorbidity in chronic Lyme disease. Arthritis Rheum 59:1742–1749. doi:10.1002/art.24314

Hatzakis A, Hadziyannis S (1984) Sex-related differences in immunoglobulin M and in total antibody response to hepatitis A virus observed in two epidemics of hepatitis A. Am J Epidemiol 120:936–942

Hirschfeld M, Kirschning CJ, Schwandner R et al (1999) Cutting edge: inflammatory signaling by Borrelia burgdorferi lipoproteins is mediated by toll-like receptor 2. J Immunol 163:2382–2386

Hodzic E, Feng S, Holden K et al (2008) Persistence of Borrelia burgdorferi following antibiotic treatment in mice. Antimicrob Agents Chemother 52:1728–1736. doi:10.1128/AAC. 01050-07

Hsu VM, Patella SJ, Sigal LH (1993) "Chronic Lyme disease" as the incorrect diagnosis in patients with fibromyalgia. Arthritis Rheum 36:1493–1500

Jarefors S, Bennet L, You E et al (2006) Lyme borreliosis reinfection: might it be explained by a gender difference in immune response? Immunology 118:224–232. doi:10.1111/j.1365-2567. 2006.02360.x

Kang I, Barthold SW, Persing DH, Bockenstedt LK (1997) T-helper-cell cytokines in the early evolution of murine Lyme arthritis. Infect Immun 65:3107–3111

Kannian P, McHugh G, Johnson BJB et al (2007) Antibody responses to Borrelia burgdorferi in patients with antibiotic-refractory, antibiotic-responsive, or non-antibiotic-treated Lyme arthritis. Arthritis Rheum 56:4216–4225. doi:10.1002/art.23135

Keane-Myers A, Nickell SP (1995) T cell subset-dependent modulation of immunity to Borrelia burgdorferi in mice. J Immunol 154:1770–1776

Kee S-J, Park Y-W, Cho Y-N et al (2012) Age- and gender-related differences in circulating natural killer T cells and their subset levels in healthy Korean adults. Hum Immunol 73:1011–1016. doi:10.1016/j.humimm.2012.07.335

Keller A, Graefen A, Ball M et al (2012) New insights into the Tyrolean Iceman's origin and phenotype as inferred by whole-genome sequencing. Nat Commun 3:698. doi:10.1038/ncomms1701

Kinjo Y, Tupin E, Wu D et al (2006) Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. Nat Immunol 7:978–986. doi:10.1038/ni1380

Klein SL (2012) Sex influences immune responses to viruses, and efficacy of prophylaxis and treatments for viral diseases. Bioessays 34:1050–1059. doi:10.1002/bies.201200099

Klein SL, Jedlicka A, Pekosz A (2010) The Xs and Y of immune responses to viral vaccines. Lancet Infect Dis 10:338–349. doi:10.1016/S1473-3099(10)70049-9

Klempner MS, Hu LT, Evans J et al (2001) Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med 345:85–92. doi:10.1056/NEJM200107123450202

Klempner MS, Baker PJ, Shapiro ED et al (2013) Treatment trials for post-Lyme disease symptoms revisited. Am J Med 126:665–669. doi:10.1016/j.amjmed.2013.02.014

Krause PJ, Narasimhan S, Wormser GP et al (2013) Human Borrelia miyamotoi infection in the United States. N Engl J Med 368:291–293. doi:10.1056/NEJMc1215469

Krupp LB, Hyman LG, Grimson R et al (2003) Study and treatment of post Lyme disease (STOP-LD): a randomized double masked clinical trial. Neurology 60:1923–1930. doi:10.1212/01.WNL.0000071227.23769.9E

Kuehn BM (2013) CDC estimates 300,000 US cases of Lyme disease annually. J Am Med Assoc 310:1110. doi:10.1001/jama.2013.278331

Lantos PM (2011) Chronic Lyme disease: the controversies and the science. Expert Rev Anti Infect Ther 9:787–797. doi:10.1586/eri.11.63

LaRocca TJ, Benach JL (2008) The important and diverse roles of antibodies in the host response to Borrelia infections. Curr Top Microbiol Immunol 319:63–103

Levy S (2013) The Lyme disease debate: host biodiversity and human disease risk. Environ Health Perspect 121:A120–A125. doi:10.1289/ehp. 121-a120

Liu N, Montgomery RR, Barthold SW, Bockenstedt LK (2004) Myeloid differentiation antigen 88 deficiency impairs pathogen clearance but does not alter inflammation in Borrelia

burgdorferi-infected mice. Infect Immun 72:2003–2010. doi:10.1128/IAI.72.6.3195-3203.2004

Ljøstad U, Mygland A (2010) Remaining complaints 1 year after treatment for acute Lyme neuroborreliosis; frequency, pattern and risk factors. Eur J Neurol 17:118–123. doi:10.1111/j.1468-1331.2009.02756.x

Lopes de Carvalho I, Núncio MS (2006) Laboratory diagnosis of Lyme borreliosis at the Portuguese National Institute of Health (1990–2004). Euro Surveill 11:257–260

Luft BJ, Dattwyler RJ, Johnson RC et al (1996) Azithromycin compared with amoxicillin in the treatment of erythema migrans. A double-blind, randomized, controlled trial. Ann Intern Med 124:785–792

Matyniak JE, Reiner SL (1995) T helper phenotype and genetic susceptibility in experimental Lyme disease. J Emerg Med 181:1251–1254

McKisic MD, Barthold SW (2000) T-cell-independent responses to Borrelia burgdorferi are critical for protective immunity and resolution of Lyme disease. Infect Immun 68:5190–5197

McKisic MD, Redmond WL, Barthold SW (2000) Cutting edge: T cell-mediated pathology in murine Lyme borreliosis. J Immunol 164:6096–6099

Meek JL, Roberts CL, Smith EVJ, Cartter ML (1996) Underreporting of Lyme disease by Connecticut physicians, 1992. J Public Health Manag Pract 2:61–65

Mehnert WH, Krause G (2005) Surveillance of Lyme borreliosis in Germany, 2002 and 2003. Euro Surveill 10:83–85

Moore J (1922a) Studies in asymptomatic neurosyphilis: II. The classification, treatment, and prognosis of early asymptomatic neurosyphilis. Bull Johns Hopkins Hosp 36:231–246

Moore J (1922b) Studies in asymptomatic neurosyphilis: III. The apparent influence of pregnancy on the incidence of neurosyphilis in women. Arch Intern Med 30:548–554. doi:10.1001/archinte.1922.00110110019002

Nadelman RB, Luger SW, Frank E et al (1992) Comparison of cefuroxime axetil and doxycycline in the treatment of early Lyme disease. Ann Intern Med 117:273–280

Nicolas J-M, Espie P, Molimard M (2009) Gender and interindividual variability in pharmacokinetics. Drug Metab Rev 41:408–421. doi:10.1080/10837450902891485

Ogrinc K, Lotrič-Furlan S, Maraspin V et al (2013) Suspected early Lyme neuroborreliosis in patients with erythema migrans. Clin Infect Dis 57:501–509. doi:10.1093/cid/cit317

Paddock C, Telford III S (2010) Through a glass, darkly: the global incidence of tick-borne diseases. Paper presented at Institute of Medicine Committee on Lyme disease and other tick-borne disease

Peters A, Lee Y, Kuchroo VK (2011) The many faces of Th17 cells. Curr Opin Immunol 23:702–706. doi:10.1016/j.coi.2011.08.007

Petzke MM, Brooks A, Krupna MA et al (2009) Recognition of Borrelia burgdorferi, the Lyme disease spirochete, by TLR7 and TLR9 induces a type I IFN response by human immune cells. J Immunol 183:5279–5292. doi:10.4049/jimmunol.0901390

Platonov AE, Karan LS, Kolyasnikova NM et al (2011) Humans infected with relapsing fever spirochete Borrelia miyamotoi, Russia. Emerg Infect Dis 17:1816–1823. doi:10.3201/eid1710.101474

Pletz MWR, Rau M, Bulitta J et al (2004) Ertapenem pharmacokinetics and impact on intestinal microflora, in comparison to those of ceftriaxone, after multiple dosing in male and female volunteers. Antimicrob Agents Chemother 48:3765–3772. doi:10.1128/AAC.48.10.3765-3772.2004

Rebman A, Crowder L, Kirkpatrick A, Aucott J (2014) Characteristics of seroconversion and implications for diagnosis of post-treatment Lyme disease syndrome: acute and convalescent serology among a prospective cohort of early Lyme disease patients. Clin Rheumatol 34:585–589

Renaud I, Cachin C, Gerster J-C (2004) Good outcomes of Lyme arthritis in 24 patients in an endemic area of Switzerland. Joint Bone Spine 71:39–43. doi:10.1016/S1297-319X(03)00160-X

Roberts BJ, Dragon JA, Moussawi M, Huber SA (2012) Sex-specific signaling through toll-like receptors 2 and 4 contributes to survival outcome of coxsackievirus B3 infection in C57Bl/6 mice. Biol Sex Differ 3:25. doi:10.1186/2042-6410-3-25

Roberts BJ, Moussawi M, Huber SA (2013) Sex differences in TLR2 and TLR4 expression and their effect on coxsackievirus-induced autoimmune myocarditis. Exp Mol Pathol 94:58–64. doi:10.1016/j.yexmp.2012.06.005

Saivin S, Houin G (1988) Clinical pharmacokinetics of doxycycline and minocycline. Clin Pharmacokinet 15:355–366. doi:10.2165/00003088-198815060-00001

Salazar JC, Pope CD, Sellati TJ et al (2003) Coevolution of markers of innate and adaptive immunity in skin and peripheral blood of patients with erythema migrans. J Immunol 171:2660–2670

Schaible UE, Kramer MD, Eichmann K et al (1990) Monoclonal antibodies specific for the outer surface protein A (OspA) of Borrelia burgdorferi prevent Lyme borreliosis in severe combined immunodeficiency (scid) mice. Proc Natl Acad Sci U S A 87:3768–3772

Schutzer SE, Berger BW, Krueger JG et al (2013) Atypical erythema migrans in patients with PCR-positive Lyme disease. Emerg Infect Dis 19:815–817. doi:10.3201/eid1905.120796

Schwarzwalder A, Schneider MF, Lydecker A, Aucott JN (2010a) Sex differences in the clinical and serologic presentation of early Lyme disease: results from a retrospective review. Gend Med 7:320–329. doi:10.1016/j.genm.2010.08.002

Schwarzwalder A, Soloski M, Lydecker A et al (2010b) The clinical and laboratory characteristics of early Lyme disease differ between men and women. Unpublished poster presentation at: Women's health 2010: the 18th annual congress, Washington, DC, 26–28 Mar 2010

Shadick NA, Phillips CB, Logigian EL et al (1994) The long-term clinical outcomes of Lyme disease. A population-based retrospective cohort study. Ann Intern Med 121:560–567

Shah K, Lee W-W, Lee S-H et al (2010) Dysregulated balance of Th17 and Th1 cells in systemic lupus erythematosus. Arthritis Res Ther 12:R53. doi:10.1186/ar2964

Sigal LH (1990) Summary of the first 100 patients seen at a Lyme disease referral center. Am J Med 88:577–581

Sigal LH, Patella SJ (1992) Lyme arthritis as the incorrect diagnosis in pediatric and adolescent fibromyalgia. Pediatrics 90:523–528

Soloski MJ, Crowder LA, Lahey LJ et al (2014) Serum inflammatory mediators as markers of human Lyme disease activity. PLoS One 9:e93243. doi:10.1371/journal.pone.0093243

Stanek G, Wormser GP, Gray J, Strle F (2012) Lyme borreliosis. Lancet 379:461–473. doi:10.1016/S0140-6736(11)60103-7

Steere AC (1989) Lyme disease. N Engl J Med 321:586–596. doi:10.1056/NEJM198908313210906

Steere AC (2001) Lyme disease. N Engl J Med 345:115–125. doi:10.1056/NEJM200107123450207

Steere AC, Angelis SM (2006) Therapy for Lyme arthritis: strategies for the treatment of antibiotic-refractory arthritis. Arthritis Rheum 54:3079–3086. doi:10.1002/art.22131

Steere A, Malawista S, Snydman D et al (1977) Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum 20:7–17

Steere AC, Hutchinson GJ, Rahn DW et al (1983) Treatment of the early manifestations of Lyme disease. Ann Intern Med 99:22–26

Steere AC, Taylor E, McHugh GL, Logigian EL (1993) The overdiagnosis of Lyme disease. JAMA 269:1812–1816

Strle K, Shin JJ, Glickstein LJ, Steere AC (2012) Association of a Toll-like receptor 1 polymorphism with heightened Th1 inflammatory responses and antibiotic-refractory Lyme arthritis. Arthritis Rheum 64:1497–1507. doi:10.1002/art.34383

Strle F, Wormser GP, Mead P, Dhaduvai K (2013) Gender disparity between cutaneous and non-cutaneous manifestations of Lyme borreliosis. PLoS One 8:e64110. doi:10.1371/journal.pone.0064110

Strle K, Stupica D, Drouin EE et al (2014) Elevated levels of IL-23 in a subset of patients with post-lyme disease symptoms following erythema migrans. Clin Infect Dis 58:372–380. doi:10.1093/cid/cit735

Tupin E, Benhnia MR-E-I, Kinjo Y et al (2008) NKT cells prevent chronic joint inflammation after infection with Borrelia burgdorferi. Proc Natl Acad Sci U S A 105:19863–19868. doi:10.1073/pnas.0810519105

Van Burgel ND, Brandenburg A, Gerritsen HJ et al (2011) High sensitivity and specificity of the C6-peptide ELISA on cerebrospinal fluid in Lyme neuroborreliosis patients. Clin Microbiol Infect 17:1495–1500. doi:10.1111/j.1469-0691.2011.03459.x

Williams PE, Harding SM (1984) The absolute bioavailability of oral cefuroxime axetil in male and female volunteers after fasting and after food. J Antimicrob Chemother 13:191–196

Wormser GP, Shapiro ED (2009) Implications of gender in chronic Lyme disease. J Women's Health 18:831–834. doi:10.1089/jwh.2008.1193

Wormser GP, Dattwyler RJ, Shapiro ED et al (2006) The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the infectious diseases society of America. Clin Infect Dis 43:1089–1134. doi:10.1086/508667

Wormser GP, Nowakowski J, Nadelman RB et al (2008) Impact of clinical variables on Borrelia burgdorferi-specific antibody seropositivity in acute-phase sera from patients in North America with culture-confirmed early Lyme disease. Clin Vaccine Immunol 15:1519–1522. doi:10.1128/CVI. 00109-08

Yin Z, Braun J, Neure L et al (1997) T cell cytokine pattern in the joints of patients with Lyme arthritis and its regulation by cytokines and anticytokines. Arthritis Rheum 40:69–79

Zöldi V, Juhász A, Nagy C et al (2013) Tick-borne encephalitis and Lyme disease in Hungary: the epidemiological situation between 1998 and 2008. Vector Borne Zoonotic Dis 13:256–265. doi:10.1089/vbz.2011.0905

Chapter 13
Effects of Sex and Maternal Immunity on Protozoan and Helminth Infections

Craig W. Roberts and William G.C. Horsnell

Abstract Protozoan and helminth parasites infect billions of people throughout the world and are responsible for significant morbidity and mortality of millions of people annually. Gender and cultural differences account for some dichotomy in the prevalence and intensity of infection between male and female humans. However, intrinsic differences in the biology, including the endocrine and immune systems, of male and female humans exert profound influence on disease pathogenesis. Generally, females are more resistant than males to many parasitic diseases, although exceptions exist, such as some cestode infections and *Toxoplasma gondii*. However, during pregnancy when a number of hormones are significantly increased and the immune system altered, females tend to be more susceptible than nonpregnant females and males to a number of parasitic infections. This is most notable for infections that rely on a helper T cell type 1 (Th1) response for resolution that is antagonized by the Th2/regulatory T (Treg) environment induced during pregnancy. As a corollary, infections that induce a strong Th1 response can disrupt pregnancy through ablating pregnancy-induced immune alterations. Some evidence is emerging that children born to mothers with parasitic infections can have lesions in their immune systems leading to tolerance or allergy as well as potential psycho-neurological changes leading to disease. There is increasing evidence that pharmacokinetics of drugs including anti-infectives can vary between the sexes. Many drugs used to treat parasitic infection (particularly protozoan infections) are far from ideal and have associated side effects. Tailored optimization of dosing regimens for men, women, and pregnant women for these drugs might be especially beneficial. New interventions optimized for sex and endocrine conditions could have greatest impact on the most disadvantaged groups in terms of susceptibility of disease including men and pregnant women.

C.W. Roberts (✉)
Professor of Parasitology, Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, United Kingdom
e-mail: c.w.roberts@strath.ac.uk

W.G.C. Horsnell
University of Cape Town, Cape Town 7700, South Africa

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_13

13.1 Introduction

In 2003, Ashford and Crewe constructed a comprehensive checklist of human parasites comprising 437 known species falling within 6 taxon (Protozoa, Trematoda, Cestoda, Nematoda, Acanthocephala, and Arthropoda). 132 of these species have been classified as "prevalent" (>5 % prevalence in at least 1 location or widespread). 69 of these prevalent parasites (dispersed through these taxa with the exception of Acanthocephala) are considered to contribute to human morbidity as pragmatically defined as "causing sufficient disease to often require therapeutics" (Ashford and Crewe 2003; Kuris 2012).

Protozoan diseases are the most prevalent with *Giardia lamblia* and *Toxoplasma gondii* having an estimated prevalence of up to 2–3 and 1–2.5 billion, respectively. However, the nematode parasites *Ascaris lumbricoides* and *Necator americanus* follow closely with 1 billion and 0.8–0.9 billion people estimated to be infected worldwide. Mortality for each of these parasites as well as *Trichomonas vaginalis* that infects approximately 15 % of the women worldwide is considered to be very low to low. However, *Plasmodium* species infect 200–300 million people and has the highest mortality rate with an estimated 627,000 deaths in 2012 (WHO http://www.who.int/mediacentre/factsheets/fs094/en/). *Entamoeba histolytica* infects 200–400 million people and accounts for 100,000 deaths each year. *Schistosoma mansoni* infects 200–300 million people and schistosomiasis due to all species of the parasite is responsible for 200,000 deaths per annum (WHO, http://www.who.int/mediacentre/factsheets/fs115/en/). *Trypanosoma* species and *Leishmania* species infect 15–20 million people and 10 million people, respectively. *Leishmania* species are responsible for 20–30,000 deaths/annum (WHO). Thus, protozoan and helminth parasites collectively are important human pathogens both in terms of prevalence and their ability to inflict human disease.

Considerable available data demonstrate differences between males and females in either or both the prevalence and severity of infection within examples of the Protozoa, Trematoda, Cestoda, Nematoda, and Arthropoda (Alexander and Stimson 1988; Roberts et al. 1996, 2001; Klein 2004; Gulgun et al. 2013). Some of these differences can be explained by differences in the behaviors between the sexes including occupation and social and cultural practices. However, there is also abundant evidence that disease pathogenesis is directly affected by the sex of the host and this often correlates with differences in the quality and quantity of immune responses. Reproduction of many of these differences in animal models under controlled conditions reinforces that the observed differences are truly mediated by the sex of the host. Furthermore, many of these differences are associated with or experimentally proven to be linked with changes in sex hormone levels. For example, some of these diseases are known to represent greater risks to pregnant women than nonpregnant women. There are now emerging data demonstrating that the pharmacokinetics of some drugs are different between males and females raising the possibility that antiparasitic drugs might vary in efficacy and side effects between the sexes (see Chap. 4). Similarly, differences between the immune

systems of males and females could profoundly influence the efficacy and undesirable reactions to vaccinations. Together, this information suggests that both prophylactic and treatment regimes might work best if they are optimized for sexes or even take into account hormonal status of the host.

13.2 Sex Differences and the Pathogenesis of Protozoan Infections and Disparity in the Success of Treatments

13.2.1 Toxoplasmosis

Toxoplasma gondii infection is acquired through ingestion of the oocyst stage, which is released by acutely infected cats to contaminate food and water, or through eating meat from chronically infected animals that harbor the tissue cyst stage. The parasite is known to infect most, if not all, warm-blooded animal species as intermediate hosts. Within these hosts, after an initial period of replication as the fast-dividing tachyzoites form, *T. gondii* transform into slow-dividing bradyzoite form that encysts in almost all tissues of the host. Most literature describes initial infection of an immunocompetent host as being associated with a general sickness response that resolves around 14 days postinfection. This is followed by a chronic, lifelong phase where the vast majority of people are generally considered to be asymptomatic (Henriquez et al. 2009). However, these broadly correct descriptions are now being challenged. Firstly, these assertions are generally based on certain, mostly clonal, genotypes of *T. gondii* present in Europe and the USA and do not apply to other clonal types that predominate in South America which tend to invoke different disease patterns. Notably, in South America with these "atypical" genotypes, disease tends to be much more severe and commonly causes ocular disease (Pfaff et al. 2014). Secondly, irrespective of the parasite genotype, the association of chronic *T. gondii* infection with neuropsychiatric disease is gaining increased attention especially in Europe and the USA (Henriquez et al. 2009). There is now robust evidence that *T. gondii* infection is associated with an increased chance of schizophrenia and emerging data on associations with memory impairment, depression, and other conditions (Henriquez et al. 2009; Arias et al. 1999; Yolken et al. 2009).

Notwithstanding the potential differences in the pathogenesis of geographically distributed *T. gondii* genotypes, infection is also known to have severe consequences in immunocompromised people and when contracted during pregnancy. Infection is universally known to be life-threatening in immunocompromised individuals, including those with AIDS, and is characterized by encephalitis, but in some cases, systemic involvement is also apparent (Torrey and Yolken 2013; Torgerson and Mastroiacovo 2013). The effects of infection during pregnancy for mother and fetus are both influenced by the stage in which infection occurs (see Sect. 13.4.2.1).

13.2.1.1 Toxoplasmosis in Male and Female

Humans

Most studies find that the incidence of infection in males and females is approximately the same. One study that examined seropositivity in France found no difference in rates between men and women under 45 years, but increased prevalence in men over 45 years (Bellali et al. 2013). Another study found that non-HIV-infected males were more likely than non-HIV-infected females to be admitted to hospital due to toxoplasmosis (Jones and Roberts 2012). Many other studies have found no difference in seropositivity in females compared with males (Daryani et al. 2014; Chiang et al. 2012; Alvarado-Esquivel et al. 2008, 2011). An early study of Scandinavian children in 1979 and a more recent study in Poland found that the incidence of *T. gondii* infection was greater in female school children than male school children. Furthermore, girls infected with *T. gondii* had poorer school performance than infected boys (Huldt et al. 1979; Mizgajska-Wiktor et al. 2013). More overt differences in disease manifestations have been reported between the sexes during acute infection. For example, lymphadenopathy was more frequently observed in males than in females under the age of 15. However, lymphadenopathy was more frequently observed in females than males in those over 25 years of age (Beverley, et al. 1976). These observations indicate that the fundamental immune response to *T. gondii* differs between males and females at different stages of their sexual maturity and implies a role for sex hormones in mediating these differences.

Most studies have found similar levels of *T. gondii* infection in males and females with HIV infection. However, toxoplasmic encephalitis was found in one study to be an AIDS-defining disease more frequently in European heterosexual females than in males (Phillips et al. 1994). Other more subtle differences between males and females infected with *T. gondii* may be present. It has been reported that in 3 cohorts of people examined from the Czech Republic, both males and females infected with *T. gondii* have reduced novelty seeking as determined by Cloninger's Temperament and Character Inventory (Flegr 2007; Flegr et al. 2003; Skallová et al. 2005; Hodková et al. 2007; Novotná et al. 2005). However, sex differences were also noted in a number of aspects examined by Raymond Cattell's 16 Personality Factors questionnaire (Cattell et al. 1993). As originally defined by Cattell, women infected with *T. gondii* had increased "warmth and rule consciousness" compared with uninfected women and seropositive men had reduced "rule consciousness," but higher "vigilance" than seronegative men. "Apprehension" was greater in all infected compared with noninfected people examined independent of sex (Henriquez et al. 2009). These studies do not define cause and effect and rather than indicating that *T. gondii* infection alters behavior, they might point to behavior attributes being a risk factor for contracting *T. gondii* infection. However, that *T. gondii* is capable of modulating aspects of mammalian behavior is well established in rodent studies. More recent data also demonstrates that some of

these behavioral changes are sex dependent in rodents. Thus, female mice but not male mice lost their natural aversion to bobcat urine. Female mice became hyperactive but male mice hypoactive. These differences between the sexes might be linked to sex-dependent differences in *T. gondii*-mediated changes to brain transcripts for a variety of genes (including some involved in olfaction and the dopamine receptor D4) and changes to monoamine levels (Gatkowska et al. 2013; Xiao et al. 2012; Flegr et al. 2011).

Together, these data suggest that the incidence of *T. gondii* infection is similar between males and females and differences that have been noticed are likely to be due to gender roles rather than sex differences. However, the immune response and disease that may result from infection might be qualitatively different between the sexes. There are no vaccines available for use in humans meaning that chemotherapy is the only option for treatment and prevention of infection. Treatment is normally a combination of anti-folates such as sulfadiazine in combination with pyrimethamine or trimethoprim and sulfamethoxazole. These drugs can be supplemented with folinic acid to rescue the host from the effects of pyrimethamine or trimethoprim that target dihydrofolate reductase (DHFR). There is little literature regarding the relative effectiveness or incidence of side effects of these drug combinations in humans. However, a recent study has found that trimethoprim and sulfamethoxazole are similarly effective in eliminating uveitis in Brazilian male and female patient with a history of eye disease (Felix et al. 2014). Based on what we know about sex differences in the pharmacokinetics and pharmacodynamics of other drugs used in the treatment of infectious diseases (see Chap. 4), future studies should more thoroughly consider whether the sexes differ in the efficacy of toxoplasmosis treatments.

Rodent Models

There is now a large body of literature demonstrating that the pathogenesis of *T. gondii* infection varies between male and female mice and is influenced by sex hormones. Early studies found that female mice infected with *T. gondii* developed more severe brain inflammation than male mice (Kittas et al. 1984; Kittas and Henry 1980). More recently, increased susceptibility of females compared with males to at least some strains of *T. gondii* has been noted in *Calomys callosus* (Franco et al. 2014). That these differences could be attributed to sex hormones was demonstrated by gonadectomy, which was found to ameliorate disease (Kittas and Henry 1979a, b). Conversely, administration of estrogens to ovariectomized female mice exacerbated disease as determined by an increase in tissue cysts in their brains (Kittas and Henry 1980). In a similar manner, estrogen treatment of gonadectomized guinea pigs made them more permissive to parasite growth relative to non-manipulated control animals (Kittas and Henry 1979a, b).

It is now known that multiple differences between male and female mice contribute to differences in disease outcome. The observation that female SCID mice (i.e., mice that are deficient in both T and B cells) are more susceptible than

male SCID mice to mortality and severe brain pathology following *T. gondii* infection implied an important role for innate immunity. Studies found these differences could be linked to the kinetics and magnitude of IL-12 and IFN-γ responses in these mice. In immunologically competent BALB/K mice, however, increased mortality in females relative to males is associated with reduced TNF-α production and early production of IL-10 (Roberts et al. 1995). C57BL6 mice infected by the oral route with *T. gondii* develop normally fatal intestinal necrosis. This was found to be worse in female mice resulting in earlier mortality than observed in male mice. In these studies, female mice had increased tachyzoite numbers and more severe necrosis in their intestines than male mice. The administration of testosterone to female mice reduced intestinal parasite load and necrosis (Liesenfeld et al. 2001). *In vitro* studies demonstrated that although progesterone is able to down-modulate nitric oxide production by macrophages, it does not ablate macrophage killing of *T. gondii* (Gay-Andrieu et al. 2002; Jones et al. 2008). This would indicate that there are multiple redundant mechanisms that contribute to the control of *T. gondii* in murine macrophages that are not all susceptible to modulation by progesterone. In reality, these limited studies performed directly with *T. gondii* do not give a full picture of the array of immune responses that are likely to be influenced by sex and pregnancy-associated hormones and consequently affect immunity to *T. gondii*. For example, sex and pregnancy-associated hormones can affect NK cells, TLR signalling, and downstream events including not only killing mechanisms but also costimulatory molecule expression. Th1 and Th2 balance, CD8 T cells, B cells, and many more aspects of immunity known to be important during *T. gondii* infection are also known to be affected by these hormones (Roberts et al. 2001; Chap. 1 of this book).

13.2.2 Leishmaniasis

13.2.2.1 Humans

Around 20 species of *Leishmania* are known to infect humans and can result in 3 main forms of disease. Visceral leishmaniasis (kala-azar) is associated with hepatosplenomegaly, infection of the bone marrow, anemia, and fever. Unless treated, it is generally fatal. Cutaneous leishmaniasis is characterized with cutaneous lesions that can heal to leave a scar. Mucocutaneous leishmaniasis is associated with the destruction of the mucous membranes and therefore affects the nose, mouth, and throat. The type of disease that develops is dependent on the parasite species and host factors including their genetics and nutrition and, pertinent to this text, their age and sex (Alexander and Stimson 1988; Alexander and McFarlane 2008; McMahon-Pratt and Alexander 2004).

The vast majority of literature reaches a consensus that in humans males are more likely to be infected with *Leishmania* and to suffer more severe disease than females (Alexander and Stimson 1988; Roberts et al. 1996, 2001; Klein 2004). This

is further reinforced by recent literature. For example, a meta-analysis of the epidemiology of visceral leishmaniasis in the Americas, attributed to *L. infantum*, found that males were more likely to develop clinical disease and to be skin test positive than females. Although children were generally found to have lower rates of infection, disease tended to be more severe than in adults (Belo et al. 2013). Cutaneous leishmaniasis in Central Amazonia has also been reported to have a similar male bias with the incidence and severity of disease being more severe in males within 2 populations of field workers and rural settlers (Soares et al. 2014). Similarly, mucocutaneous leishmaniasis was found to be 1.7 times more likely to occur in males than females (Machado-Coelho et al. 2005). In spite of this wealth of epidemiological data, there is very little known about differences in the immune responses of humans to *Leishmania*. One study demonstrated that in humans with *L. mexicana* infection, males produce greater quantities of IgE but have reduced delayed-type hypersensitivity (DTH) responses than females (Lynch et al. 1982). This would suggest that males tend toward a Th2-biased response, but females tend toward a Th1 response following infection with this parasite. However, murine models of disease provide some insights and imply a much more complex situation is at play.

13.2.2.2 Rodent Models

While some murine studies are consistent with the above observation in humans, others would appear to contradict them. Thus, in some strains of mice, including BALB/c and DBA/2 mice, males are more susceptible than females to systemic infection with *L. major*. The role of both male and female sex hormones is evident as BALB/c males could be rendered resistant by gonadectomy and females rendered susceptible by administration of testosterone (Mock and Nacy 1988). Similarly, male DBA/2 mice are more susceptible than female mice to *L. mexicana*-induced cutaneous disease (Alexander 1988). Resistance of females is associated with induction of a Th1 response as evident by the production of IFN-γ. Gonadectomy increases the susceptibility of female DBA/2 mice to cutaneous infection but increases the resistance of males (Alexander 1988; Alexander, and Stimson 1988; Satoskar et al. 1998; Satoskar and Alexander 1995). More recent work in BALB/c mice confirms that sex-mediated differences in Th1/Th2 balance are important as female IL-4Rα-deficient mice (which are thus unable to respond to IL-4 or IL-13) heal following infection with *L. mexicana*, but male mice do not (Bryson et al. 2001). *In vitro* studies have demonstrated that progesterone inhibits macrophage nitric oxide production and reduces the killing of *L. donovani* (Jones et al. 2008). The extent of the interaction of sex hormones with the immune system and how this ultimately influences immunity to *Leishmania* is likely to be more complex than these studies suggest and include many other general mechanisms already reviewed in Chap. 1 of this book.

13.2.3 Malaria

Malaria in humans can be caused by *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, or *Plasmodium ovale*. Much of the historic epidemiological data gathered for malaria was not sex disaggregated, but some of the literature suggest that the incidence of infection is similar between male and female humans. However, more recent data demonstrates the incidences of *P. falciparum* or *P. vivax*, at least in some populations, are greater in males compared with females (Venugopalan et al. 1997; Lin et al. 2009; Haque et al. 2011; Cucunubá et al. 2013; Pathak et al. 2012; Tareen et al. 2012). In contrast, females appear to be at greater risk than males of death due to *P. falciparum* (18.4 % versus 7.64 %) (Kochar et al. 1999). These data are of course complicated and open to interpretation as gender differences in work patterns; access to preventive measures, such as insecticide-impregnated bed nets; and health care also vary.

A murine model of malaria that relies upon C57BL/10 mice infected with *Plasmodium chabaudi* has robustly demonstrated that male mice are more susceptible than females to infection (Benten et al. 1991, 1992a, b, 1993, 1997, 1998, 1999). This susceptible phenotype exhibited by male mice is reversed by castration indicating a detrimental role for testosterone. This hypothesis was confirmed by administration of testosterone to females which ablated their resistance to infection. The effect of testosterone on females is profound and long-lasting as administration prior to infection followed by an extended 9 weeks period without testosterone that allowed testosterone levels to return to baseline still resulted in increased susceptibility. This testosterone-mediated susceptibility to *P. chabaudi* infection was found to be associated with upregulation of a novel transmembrane protein termed IAP38 (Krucken et al. 1999). Testosterone was also found to upregulate the expression of a number of miRNA (miR-22, miR-690, miR-122, let-7A, miR-30D, and let-7D) and consequently downregulate a number of gene products including ERalpha and aromatase. Further studies have demonstrated that testosterone has long-lasting and widespread, organ-specific effects on lincRNA, miRNAs, and TLR expression that may account for some of the observed effects it has on the susceptibility of mice to *P. chabaudi* (Krucken et al. 1997). Early studies demonstrated that the effects of testosterone in this model are not dependent on classical androgen receptors because androgen receptor antagonist was unable to ablate the observed effects. Moreover, susceptibility can be transferred by T cells that are known to lack classical androgen receptors, but instead express surface androgen receptors (Benten et al. 1992a, b, 1997). Together, these data suggest that testosterone acts on the surface receptors of T cells to modulate miRNA and lincRNA, which regulate the expression of other gene products that modulate immune function.

13.2.4 Amoebiasis

Entamoeba histolytica is the causative agent of amoebiasis in humans. Although it infects 200–400 million people, only about 40–50 million people develop colitis or amoebic liver abscess (ALA) of which around 100,000 die as a result each year. ALA is predominantly a disease of males, which comprise approximately 80 % of all cases. The number of cases is low in males and females under 20 years of age, but increases sharply thereafter in males, reaching their maximum in the 36–40-year age group coinciding with peak testosterone levels (Blessmann et al. 2002; Bernin and Lotter 2014). The number of cases in woman peaks post menopause, but never approaches that of men (Blessmann et al. 2002).

A detrimental role for testosterone is also evident in murine studies as administration of this hormone can promote parasite replication and abscess development in normally resistant female mice. The ability of female mice to control infection appears to be due to NKT cell-mediated control of parasite numbers through the production of IFN-γ, which is ablated in female mice by administration of testosterone. In contrast, gonadectomized male mice are resistant to abscess development and have enhanced NKT cell production of IFN-γ relative to intact control mice (Lotter et al. 2013). There is also evidence that tissue damage in male mice is dependent on the production of TNF-α by Kupffer cells and monocytes (Helk et al. 2013).

13.3 Sex Differences and the Pathogenesis of Helminth Infections and Disparity in the Success of Treatments

Host gender or sex can strikingly influence immunity to and the prevalence of helminth infections. Typically, males demonstrate increased susceptibility to and prevalence to/of nematode infections (Klein 2004). However, this scenario may be reversed for cestode/trematode infections, in which both murine and clinical studies indicate that females may be more susceptible than males (Nava-Castro et al. 2012).

Heightened susceptibility of males to nematode infections is frequently, but not always, associated with testosterone. Experimental mouse infections with *Trichuris muris* have elegantly demonstrated that testosterone inhibits protective immunity to the parasite (Hepworth et al. 2010; Hepworth and Grencis 2009). In response to *T. muris*, raised testosterone reduced disease resolving TH2 cytokine responses, especially IL-13, whereas removal of the primary source of testosterone via castration enhanced host immunity to *T. muris* (Hepworth et al. 2010). Treatment of cells pulsed with *T. muris* antigen with 17-β oestradiol enhanced TH2 cytokine (IL-13 and IL-4) secretion by these cells. However, removal of ovaries had no effect on host ability to resolve infection but still enhanced goblet cell hyperplasia in IL-4KO mice. In addition, these studies also demonstrate sex differences in NK cell responses. In female mice, NK cells may be an important source of IL-13

during *T. muris* infection (Hepworth et al. 2010; Hepworth and Grencis 2009). Recent analysis of genetic differences underlying male susceptibility suggests an autosomal quantitative trait locus on chromosome 5 of male mice may drive an enhanced IFN-γ response that could impair induction of protective TH2 responses (Hayes et al. 2014). This finding represents an initial step toward delineating chromosomal characteristics that drive the differential gene expression patterns that underlie sex differences in susceptibility to this nematode infection.

Levels of testosterone can vary by social rank, which can affect susceptibility to nematode infection. Males with higher social rank and elevated corticosterone concentrations have impaired immunity against infection with *Heligmosomoides polygyrus* (Barnard et al. 1988). The likelihood of sex hormones influencing enhanced resistance in females has been suggested by studies showing that both progesterone and estradiol can inhibit the development of *T. spiralis in vitro* (Hernández-Bello et al. 2011).

Increased susceptibility of males to nematode infections is also a feature of filarial infections; testosterone, however, does not appear to be a major contributing factor because neither castration of male mice (Rajan et al. 1994a, b) nor endogenous elevations of testosterone influence worm burden (Ganley and Rajan 2001). It also appears that neither T- nor B-cell responses are necessary for male susceptibility to filarial nematode infections because *Brugia malayi* worm burdens are comparable between wild-type and SCID male mice (Rajan et al. 1994a, b). Differences in the kinetics of parasite progression through its life cycle and demonstrated by the time when molting occurs suggest that the host environment in male mice may be more permissive to the parasite (Rajan et al. 1994a, b). Precise mechanisms driving sex-associated susceptibility to filarial nematodes are currently incompletely understood.

Clinical studies support these experimental findings in murine models by suggesting that the sex of the host affects the prevalence and outcome of parasitic nematode infections. Hookworm infections, for example, appear to be more common in males (Behnke et al. 2000; Pullan et al. 2008) as is schistosomiasis (Pullan et al. 2008). Filarial nematode infections show a higher prevalence in males (22.9 %) than females (12.6 %) in both rural (Chesnais et al. 2014) and urban endemic regions (Mwakitalu et al. 2014). Whether differences in the prevalence of nematode infections between men and women reflect differential host immunity, hormone levels, behavior, or a combination of factors must be empirically evaluated. When it comes to treating helminth infections, sex-specific host responses to anti-helminthic drugs do not appear to occur (Vercruysse et al. 2011).

For cestode infections, females show a reduced ability to control infection as compared with males. This has been modeled using the murine parasite *Taenia crassiceps*. Female mice show higher rates of parasitism than males and chronic infection can actually feminize male mice (Larralde et al. 1995). Experimental removal of gonads can reverse female susceptibility to *T. crassiceps* (Huerta et al. 1992). Enhanced parasite growth in females may result in part from parasite utilization of host estrogens, which are in higher concentrations in females, to enable cystircerci development (Huerta et al. 1992). Although females produce a

stronger antiparasitic macrophage response than males to *T. crassiceps*, whether this mediates protection is not known (Togno-Peirce et al. 2013). These findings are supported by some clinical and veterinary studies of *Taenia solium*, which also demonstrate higher rates of cysticercosis in female compared with male hosts (Morales-Montor et al. 2004).

For schistosome infections, elevated testosterone levels in male mice appear to drive resistance to experimental infections. While testosterone is related to reduced worm burdens, it has no major effects on egg deposition in the liver (Nakazawa et al. 1997). Clinically, human males show higher rates of infection with schistosomes (Pullan et al. 2008). For filarial infection, data suggest that gender-associated behavior (e.g., men are more likely to hunt and fish and therefore enter areas with higher risk of transmission) as opposed to sex differences in immunity may be the key contributor to increased levels of infection in males (Pinot de Moira et al. 2010).

13.4 Pregnancy and Infection

13.4.1 The Effect of Maternal Helminth Infection on Pregnancy and Health of the Infant

High helminth infection rates among pregnant women in areas of helminth endemicity are widely reported (Hotez and Kamath 2009; Labeaud et al. 2009; Adegnika et al. 2007). This exposure to helminth infections will have both local and systemic effects on the health of the mother, ranging from anemia to hepatic damage, depending on the parasites to which the pregnant woman is exposed. How significant this maternal pathology and/or changes in maternal immunity may be on the newborn child is unclear.

Anemic effects, which would most likely result from heavy hookworm infection, could exacerbate underlying maternal nutritional stress, which can be common in endemic areas (Hotez et al. 2008). The end result of this may be reduced birth weight in infants. A number of studies have addressed this question in endemic areas, but findings are mixed (Fairley et al. 2013; Larocque et al. 2006). Difficulty in establishing how maternal infections influence birth weight is principally due to the high levels of coinfection, making identifying helminth-specific influences difficult (McClure et al. 2014). It should, however, be appreciated that maternal hookworm infections could cause increased infant anemia at birth. The long-term consequences of this are not known but may contribute to purported effects on, for example, cognitive development.

The more acute host pathologies of patent *Schistosoma* spp. infections would be expected to have more striking effects on infant health. However, maternal exposure can also confer protection of offspring against a subsequent infection, at least in mouse models (Montesano et al. 1999a, b). Current clinical studies in the

Philippines indicate that maternal schistosome infections can lead to reduced birth weight and this may be associated with placental inflammation and the onset of parasite-associated fibrotic responses in the mother (Kurtis et al. 2011; McDonald et al. 2013, 2014).

Should maternal helminth infections negatively affect the outcome of pregnancy, it is important that we understand the safety of treating mothers with anti-helminthic drugs because such treatment is not routinely recommended (Haider et al. 2009). Maternal anti-helminthic treatments with mebendazole and praziquantel can be given during pregnancy and may lead to some increase in newborn weight (Larocque et al. 2006). However, studies also show that maternal deworming strongly associates with an increased prevalence of allergy (e.g., eczema) in children (Mpairwe et al. 2011; Ndibazza et al. 2012). This would be in line with many recent studies showing that the helminthic "macro-biome" can be strikingly protective against autoimmune/allergic disease (McSorley and Maizels 2012). Current data on maternal helminth infection influencing allergy is limited but ongoing research efforts in Africa, South America, and South East Asia will advance our clinical understanding of this potential influence on infant health.

A research need therefore exists to add clarity to our understanding of how maternal helminth infection impacts on fundamental markers of a successful pregnancy, such as birth weight, subsequent growth, and cognitive development. A comprehensive understanding of the safety of antenatal anti-helminthic treatment also needs to be addressed. The potential for maternal helminth infection reducing the risk of infants developing allergy is an important research question, which may have far-reaching consequences for understanding the high rates of autoimmune disease in areas no longer endemic for helminth infections.

13.4.2 Infection with Protozoans Can Have Profound Adverse Effects on Pregnancy

The immunology of healthy pregnancy and how this can influence systemic immunity is discussed in Chap. 3. Overall, successful pregnancy is dependent on control of immunological conditions in fetoplacental tissues. This includes expansion of Treg and Th2 cells in the uterus and placenta and production of their signature cytokines, including IL-4, IL-5, and IL-10. NK cell function is also tightly regulated through production of progesterone-induced inhibitory factor (PIBF) by CD8 T cells. In contrast, Th1 and Th17 expansion have been associated with fetal loss. In murine models, administration of Th1 products such as IFN-γ and IL-2 has proven abortogenic (Druckmann and Druckmann 2005). In addition, inflammatory mediators including TNF-α or nitric oxide, associated with innate immune cell stimulation or Th1 cell activation, also have adverse effects on pregnancy (Athanassakis et al. 1999) These observations provide a framework to test and understand the

potential of infections to affect pregnancy and indeed for pregnancy to influence the course of infection.

A number of protozoans are known to be congenitally transmitted and include *Toxoplasma gondii*, *Trypanosoma cruzi*, *Plasmodium* species, African trypanosomes, and *Leishmania* species. Notably, each of these parasites is associated with the induction of innate immune mediators including TNF-α and NO by interaction with TLRs. Protective immunity to all these parasites is at least in part dependent on the induction of a Th1 response. As such, these parasites have the ability to disrupt pregnancy indirectly through modulating the immune system. Some can also cause damage to the placenta providing another route to disrupt pregnancy. Congenital infection can cause low birth weight and long-term, possibly lifelong illness. Congenital infection or *in utero* exposure can also cause imprinting of the neonate immune system and affect their ability to respond to the parasite later in life. Congenital exposure to *Trypanosoma cruzi* has been demonstrated to affect the immune response to heterologous pathogens (Dauby et al. 2009). How widespread such heterologous effects could be, or if similar effects for different congenital infections are common, remains to be determined. Certainly, the effects of maternal helminth infections are known to affect the immunity of the infant (see below). Below, we provide some examples with toxoplasmosis, malaria, and leishmaniases. The subject of congenital transmission of parasitic infections has been reviewed (Carlier et al. 2012).

13.4.2.1 Toxoplasmosis

Murine studies have demonstrated that the ability of *T. gondii* to stimulate a robust innate immune response with NK cell activation, Th1 cell expansion, and inflammatory cytokines has detrimental effects on gestation. Furthermore, pregnancy-induced immunomodulation would appear to increase the susceptibility of mice to *T. gondii* infection. How the interplay of each of these scenarios impacts pregnancy depends on the timing of infection during pregnancy. Murine studies also demonstrate that *T. gondii* infection acquired during the first trimester generally induces abortion (Roberts and Alexander 1992). Immunological mechanisms including the production of TNF-α and nitric oxide and ablation of Th2 bias in favor of Th1 bias, all induced by *T. gondii* infection, are likely to play a role in this abortion (Roberts et al. 1996, 2001). Recent studies report an increase in Th17 cells and a decrease in Treg cells in the placenta of *T. gondii* infected relative to control placenta from noninfected mice (Saito et al. 2010, 2011). In contrast, infection acquired in the second or third trimester, when a strong Th2/Treg bias is established, normally goes to term but might result in congenital transmission (Roberts and Alexander 1992). The pregnancy-induced modulation of the immune system might actually facilitate parasite survival and thus transmission. Notably, pregnant mice are also more susceptible to *T. gondii*-induced mortality and have depressed IFN-γ production compared with nonpregnant control mice (Shirahata et al. 1992).

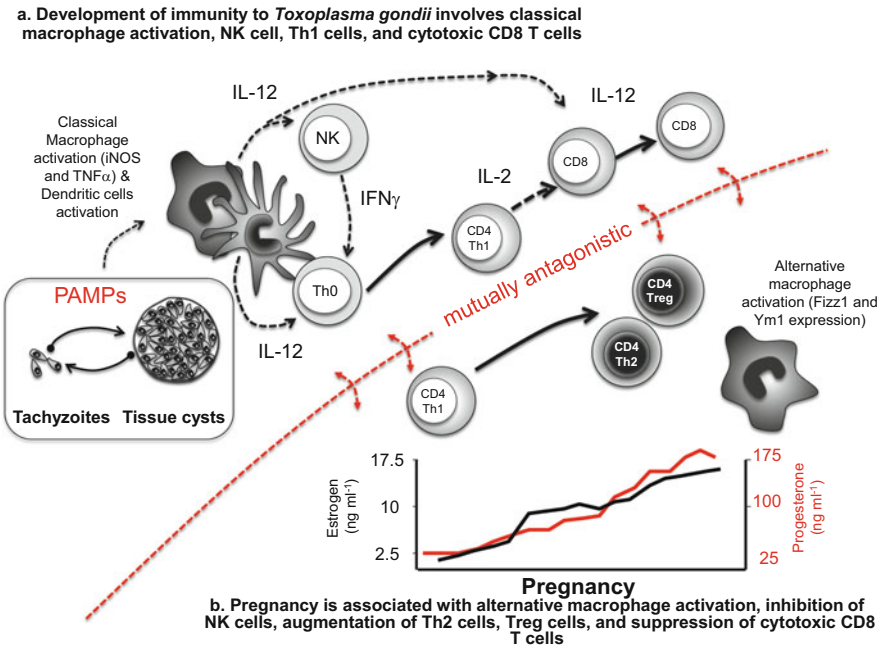

Fig. 13.1 Development of protective immunity and successful pregnancy are mutually antagonistic. (**a**) Immunity to *T. gondii* is dependent on a robust innate immune response induced by a number of *T. gondii* PAMPs (Pathogen-Associated Molecular Patterns), resulting in the production of IL-12 and TNF-α and the activation of NK cells and production of IFN-γ. Together, this immunological environment induces the expansion of Th1 cells and cytotoxic CD8 T cells. (**b**) The success of pregnancy is dependent on endocrine changes including increased estrogen and progesterone levels that influence immunity. This leads to alternative macrophage activation; inhibition of NK cells, Th2 cells, and Treg cells; and suppression of cytotoxic CD8 T cells (diagram expands and adapts some concepts previously used in Henriquez et al. 2009)

In humans, epidemiological observations are consistent with murine studies. The risk of abortion following *T. gondii* infection is greater in the first trimester than the second trimester, which is greater than the third trimester. In contrast, the risk of congenital transmission is high (65 %) in third trimester, intermediate in the second trimester (54 %), and low in the first trimester (24 %) (Boyer and McLeod 1998; Roberts et al. 1996, 2001). These observations are consistent with the hypothesis that control of *T. gondii* and successful pregnancy are antagonistic and incompatible (Fig. 13.1).

The dogma of historical literature is that humans harboring a chronic infection do not transmit parasites to their offspring. However, increasing numbers of studies now demonstrate that this is not entirely true and parasite transmission occurs in a small number of cases sometimes, but not always, associated with other underlying immunosuppression conditions (Vogel et al. 1996; Kodjikian et al. 2004; Bachmeyer et al. 2006 ; Boyer et al. 2005; Silveira et al. 2003; Andrade et al. 2010). A retrospective study that examined 18 females with chronic

T. gondii infection through 35 pregnancies reported seven cases of ocular toxoplasmosis due to disease reactivation (Garweg et al. 2005). Collectively, these studies indicate that pregnancy-induced alteration to systemic immunity can cause reactivation of chronic disease in at least some individuals.

13.4.2.2 Malaria

In most, but not all, studies, pregnant women have been found to have an increased risk of malaria infection and development of more severe symptoms than nonpregnant women (Menendez 1995; Kochar et al. 1999; Eisele et al. 2012; Barcus et al. 2007; Steketee 2014). Generally, primigravidae women and their fetuses are at greater risk of increased complications than multigravidae women (Menendez 1995). Pregnant women tend to suffer from increased anemia and in most studies have been found to be more likely to die. *P. falciparum* alone has been estimated to be responsible for around 10,000 maternal deaths/annum (Steketee et al. 2001).

Malaria also has an adverse effect on pregnancy and is associated with abortion, preterm birth, and low birth weight (Menendez 1995; Eisele et al. 2012). It is now known that a unique population of infected erythrocytes sequester in that placenta. These "placental-type" parasites bind chondroitin sulfate A in the placenta through a variant of the *Plasmodium falciparum* erythrocyte membrane 1 protein 1 (PfEMP1), termed variant surface antigen 2-CSA (VAR2CSA) (Ataíde et al. 2013). Their sequestration and subsequent monocyte infiltration into the placental intervillous spaces are likely to be responsible for many of these adverse effects on the fetus. However, there are also immunological changes in the placenta that are known to have adverse effects on pregnancy. Adverse outcomes have been associated with increased Th1 responses; IL-1β, IFN-γ, and IL-2 production; and high levels of placental levels of TNF-α (reviewed, Rogerson et al. 2007). It would seem reasonable that these immunological effects would be accentuated by sequestration of infected erythrocytes and monocyte infiltration. Antibodies develop against VAR2CSA during pregnancies and would appear to account for the better prognosis of pregnancies in multigravida women (Ataíde et al. 2013).

In a meta-analysis of 32 studies, it was found that full treatment of women during their first or second trimester of pregnancy with antimalarial intermittent preventive therapy during pregnancy (IPTp) or insecticide-treated mosquito nets (ITNs) significantly reduced the risk of neonatal mortality compared with nontreated women (Eisele et al. 2012). In another meta-analysis, treatment had clear benefits for maternal and neonatal health as demonstrated by a reduction in maternal anemia by 38 %, low birth weight by 43 %, and perinatal mortality by 27 % (Desai et al. 2007).

13.4.2.3 Leishmaniasis

In mice, *Leishmania major* infection can adversely affect pregnancy and pregnancy can alter immunity and the progression of Leishmaniasis in adult mice. Thus, lymphocytes from pregnant mice produce increased amounts of IL-4, IL-5, and IL-10 and are more permissive to parasite growth, as measured in their footpads, compared with nonpregnant mice. This apparent skewing of the immune response to a Th2 phenotype in pregnant mice is reinforced by the increase in IgG2a and decrease in IgG1 in these mice compared with nonpregnant mice. Furthermore, pregnant C57BL/6 females had fewer implantations and increased numbers of resorptions compared with pregnant females that were not infected. In contrast, BALB/c mice that typically mount a strong Th2 response to *L. major* infection and are therefore susceptible to severe disease did not exhibit reduced numbers of implantations (Krishnan et al. 1996a, b). These results have clear implications for the success of conception and pregnancy in humans and would support that host genetics play an important role.

The extent to which leishmaniases affect conception and pregnancy in humans is not well studied. A review of 26 cases of cutaneous leishmaniases in pregnant woman in Brazil found that lesions in pregnant women were much larger than those in nonpregnant age-matched women (Morgan et al. 2007). The mechanisms accounting for this observation was not explored. However, a recent report documents 2 cases in a short period where the cutaneous lesions of woman with *Leishmania braziliensis* became enlarged. Immunological measurements were consistent with a pregnancy-induced bias of the immune response toward Th2. Thus, lymphocytes from pregnant woman produced less IFN-γ than those from nonpregnant infected controls. The frequency of IL-10 producing lymphocytes upon stimulation with parasite antigen was increased in samples from pregnant but not nonpregnant woman. Arginase levels were also increased in pregnant woman, which the authors hypothesized might affect T-cell proliferation. Postpartum, many of these apparently pregnancy-altered parameters were reversed or even augmented relative to nonpregnant controls. Furthermore, iNOS was raised and parasite numbers decreased postpartum relative to during pregnancy (Conceição-Silva et al. 2013).

13.5 Influence of Maternal Infection or Congenital Transmission on Immunity in Infants Born to Infected Mothers

13.5.1 Helminth Infection

Helminth infections are rarely transmitted congenitally (reviewed Carlier et al. 2012), but maternal infection may influence a child's ability to respond to

an unrelated infection and, by association, may alter the efficacy of childhood vaccines. A relatively large number of studies have addressed the impact of maternal helminth infections on immunity in children.

Studies in the 1980s demonstrated the presence of parasite-specific IgE and IgM in cord blood, immunoglobulin isotypes that do not normally cross the placenta, demonstrating what may be considered an important mechanism of prenatal sensitization from mothers with chronic schistosome (Eloi-Santos et al. 1989) and filarial (Agarwal et al. 1986; Weil et al. 1983) infections.

The immunological ramifications of such *in utero* stimulation have been demonstrated in studies showing helminth-specific responses in young children who are unlikely to have experienced a patent helminth infection. For example, helminth-specific B-cell responses and helminth antigen-specific IgG titres have been shown in children whose mothers had chronic helminth infections during pregnancy (King et al. 1998). Additionally, maternal infection with *Ascaris lumbricoides* has also been associated with cord blood *A. lumbricoides*-specific T-cell cytokine production (Guadalupe et al. 2009). Of interest is that this maternal helminth exposure may increase a child's susceptibility to *Ascaris* infection (Mehta et al. 2012). However, experimental studies also demonstrate a striking antibody-dependent transfer of protective immunity against helminths in offspring (Harris et al. 2006).

Chronic maternal helminthiasis may also affect immune responses to unrelated antigens. Hypothetically, helminth promotion of Th2 cytokine production and induction of Treg cell responses should impair inflammatory cytokines (Elias et al. 2008; Figueiredo et al. 2010; van Riet et al. 2007). Such changes have been suggested to diminish childhood vaccine efficacy and lead to more rapid progression of and increased susceptibility to unrelated infections (reviewed here (van Riet et al. 2007)). For example, helminth-specific immunity acquired *in utero* by infants born to infected mothers can persist into childhood and lead to an impaired protection-associated IFN-γ immune responses to BCG vaccination (Malhotra et al. 1999). However, no clinical studies have demonstrated that maternal helminth exposure results in increased susceptibility to mycobacterial infection or disease.

Perhaps surprisingly, the most compelling evidence to date may indicate that potential routine treatment (i.e., albendazole) in endemic areas of pregnant mothers for helminth infection may need to be reconsidered. Findings in Uganda have indicated that albendazole treatment has no effect on childhood vaccine efficacy (Webb et al. 2011) but increases the likelihood of eczema (Mpairwe et al. 2011; Ndibazza et al. 2012).

These findings are in agreement with data demonstrating that experimental helminth infections protect against a range of autoimmune diseases including allergic disorders (Fallon and Mangan 2007; Hewitson et al. 2009). This protection is dependent on helminth-induced regulatory immune cell populations, especially Treg cell populations (Grainger et al. 2010). Clinical studies demonstrating enhanced cord blood IL-10 relating to increased childhood susceptibility to *A. lumbricoides* may also indicate possible protection against allergy. However, to date, no studies have shown that maternal exposure to helminths can protect offspring from allergy.

It may be that maternal exposure to helminths is an important contributor to our immune education and protection against the onset of autoimmune disease, allergy, and even infection. However, we should be cautious in making these assumptions. Many helminthic infections occur in areas of high population density and poor sanitation. This leads to high parasite burdens and host pathology. This may well lead to negative influence on pregnancy and child health. Currently, our understanding is poor and a combined effort by clinical and experimental researchers to dissect how maternal infections influence mother and child health is needed.

13.5.2 Toxoplasmosis

Humans congenitally infected with *T. gondii* can be born with a range of disease manifestations from severe life-threatening hydrocephalus to apparently asymptomatic at birth. However, even those with mild-to-asymptomatic disease are likely to be plagued with chronically recurring ocular disease for their lifetime. This is presumably due to congenitally infected humans having altered immune responses to *T. gondii* relative to the vast majority of those that acquire disease postpartum or as adults and for the most part develop protective immunity and do not suffer recurring disease (Roberts et al. 2014). Peripheral blood lymphocyte blastogenic responses as well as IFN-γ and IL-2 production to *Toxoplasma* lysate antigen have been noted to be reduced in congenitally infected infants compared with recently or chronically infected adults (McLeod et al. 1985: McLeod et al. 1990). αβT cells would appear to exhibit a degree of anergy to *T. gondii* antigen or *T. gondii*-infected cells for an extended period of time. In contrast, γδT cells although anergic soon after birth overcame this when the infant is approximately 1-year-old and were able to respond to *T. gondii* as normal with proliferation and production of IFN-γ (Hara et al. 1996). A degree of immunological unresponsiveness and, as a consequence, disease reactivation would appear to persist for life in congenitally infected people and illustrate the need for more work in this area.

13.6 Conclusion and Areas of Future Research Required

After accounting for gender-influenced, cultural and behavioral differences, there is clear evidence that the pathogenesis including severity of many parasitic infections varies between the sexes. The role of hormones in regulating certain aspects of the immune response and thus disease progression is convincing especially through animal models. Two disadvantaged groups emerge in terms of susceptibility to parasitic diseases, men and pregnant women who share a disproportionate burden of disease severity. There are no approved vaccines for the parasites discussed in this chapter, but vaccines used to prevent at least some bacterial or viral pathogen infections have been demonstrated to vary in their efficacy when used in males and

females. This highlights the need to test putative vaccines under development for parasitic infections in both sexes.

Studies have demonstrated that the pharmacokinetics of drugs can vary considerably between males and females (see Chap. 4). However, very few studies have tested whether the relative efficacy of antiparasitic drugs differs between males and females. Such testing could not only increase the potential efficacy of currently used drugs but also limit adverse reactions. This could have a significant impact as many drugs used for treatment of parasitic infections have significant side effects and can themselves be dangerous.

The ability of infections in general but particularly parasitic infections that are so widespread to affect maternal and neonatal health and potentially have long-lasting effects on people born to affected mothers is a significant concern and an important area of future research. The importance of tackling the issue of maternal infections effectively is emphasized with recent studies that demonstrate potentially lifelong changes to progeny of infected mothers even in the absence of congenital infection. Thus, there is robust evidence of an association between maternal infections and the development of schizophrenia and autism. Animal models have demonstrated that maternal immune activation is associated with behavioral changes in the progeny and evidence is growing in humans that maternal immune activation might be associated with an array of behavioral syndromes and diseases later in life including Alzheimer's and Parkinson's diseases (Knuesel et al. 2014).

References

Adegnika AA, Agnandji ST, Chai SK, Ramharter M, Breitling L, Kendjo E, Issifou S, Yazdanbakhsh M, Kombila M, Kremsner PG (2007) Increased prevalence of intestinal helminth infection during pregnancy in a Sub-Saharan African community. Wien Klin Wochenschr 119:712–716

Agarwal M, Prasad GB, Harinath BC, Bhatia BD (1986) Transplacental transfer of filarial infection. Indian Pediatr 23(3):169–174

Alexander J (1988) Sex differences and cross-immunity in DBA/2 mice infected with *L. mexicana* and *L. major*. Parasitology 96:297–302

Alexander J, McFarlane E (2008) Can type-1 responses against intracellular pathogens be T helper 2 cytokine dependent? Microbes Infect 10:953–959. doi:10.1016/j.micinf.2008.07.038

Alexander J, Stimson WH (1988) Sex hormones and the course of parasitic infection. Parasitol Today 4:189–193

Alvarado-Esquivel C, Cruz-Magallanes HM, Esquivel-Cruz R, Estrada-Martínez S, Rivas-González M, Liesenfeld O, Martínez-García SA, Ramírez E, Torres-CastorenaA CA, Dubey JP (2008) Seroepidemiology of *Toxoplasma gondii* infection in human adults from three rural communities in Durango State, Mexico. J Parasitol 94:811–816

Alvarado-Esquivel C, Estrada-Martínez S, Liesenfeld O (2011) *Toxoplasma gondii* infection in workers occupationally exposed to unwashed raw fruits and vegetables: a case control seroprevalence study. Parasit Vectors 4:235

Andrade GM, Vasconcelos-Santos DV, Carellos EV, Romanelli RM, Vitor RW, Carneiro AC, Januario JN (2010). Congenital toxoplasmosis from a chronically infected woman with

reactivation of retinochoroiditis during pregnancy. J Pediatr (Rio J) 86:85–88. doi: doi:10.2223/JPED.1948

Arias I, Sorlozano A, Villegas E, de Dios LJ, McKenney K, Athanassakis I, Aifantis I, Ranella A, Giouremou K, Vassiliadis S (1999) Inhibition of nitric oxide production rescues LPS-induced fetal abortion in mice. Nitric Oxide 3:216–224

Ashford RW, Crewe W (2003) Parasites of Homo sapiens: and annotated checklist of the protozoa, helminths and arthropods for which we are home. CRC Press, New York

Ataíde R, Mayor A, Rogerson SJ (2013) Malaria, primigravidae, and antibodies: knowledge gained and future perspectives. Trends Parasitol 30:85–94. doi:10.1016/j.pt.2013.12.007

Athanassakis I, Aifantis I, Ranella A, Giouremou K, Vassiliadis S (1999) Inhibition of nitric oxide production rescues LPS-induced fetal abortion in mice. Nitric Oxide 3(3):216–224

Bachmeyer C, Mouchnino G, Thulliez P, Blum L (2006) Congenital toxoplasmosis from an HIV-infected woman as a result of reactivation. J Infect 52:55–57

Barcus MJ, Basri H, Picarima H, Manyakori C, Sekartuti EI, Bangs MJ, Maguire JD, Baird JK (2007) Demographic risk factors for severe and fatal *vivax* and *falciparum* malaria among hospital admissions in northeastern Indonesian Papua. Am J Trop Med Hyg 77:984–991

Barnard CJ, Behnke JM, Gage AR, Brown H, Smithurst PR (1988) The role of parasite-induced immunodepression, rank and social environment in the modulation of behaviour and hormone concentration in male laboratory mice (*Mus musculus*). Proc Biol Sci 265(1397):693–701

Behnke JM, De Clercq D, Sacko M, Gilbert FS, Ouattara DB, Vercruysse J (2000) The epidemiology of human hookworm infections in the southern region of Mali. Trop Med Int Health 5:343–354

Bellali H, Pelloux H, Villena I, Fricker-Hidalgo H, Le Strat Y, Goulet V (2013) Prevalence of toxoplasmosis in France in 1998: is there a difference between men and women? At what age do children become infected? Rev Epidemiol Sante Publique 61:311–317

Belo VS, Werneck GL, Barbosa DS, Simões TC, Nascimento BW, da Silva ES, Struchiner CJ (2013) Factors associated with visceral leishmaniasis in the americas: a systematic review and meta-analysis. PLoS Negl Trop Dis 7:e2182

Benten WP, Bettenhaeuser U, Wunderlich F, Van Vliet E, Mossman H (1991) Testosterone-induced abrogation of self-healing of *Plasmodium chabaudi* malaria in B10 mice:mediation by spleen cells. Infect Immun 1991:4486–4490

Benten WP, Wunderlich F, Mossman H (1992a) *Plasmodium chabaudi*: estradiol suppresses acquiring, but not once acquired immunity. Exp Parasitol 75:240–247

Benten WP, Wunderlich F, Mossman H (1992b) Testosterone-induced suppression of self-healing *Plasmodium chabaudi*: and effect not mediated by androgen receptors? J Endocrinol 135:407–413

Benten WP, Wunderlich F, Hermann R, Kuhn-Velten WN (1993) Testosterone-induced compared with oestradiol-induced immunosuppression against *Plasmodium chabaudi* malaria. J Endocrinol 139:487–494

Benten WPM, Ulrich P, KuhnVelten WN, Vohr HW, Wunderlich F (1997) Testosterone-induced susceptibility to Plasmodium chabaudi malaria: persistence after withdrawal of testosterone. J Endocrinol 153:275–281

Benten WPM, Lieberherr M, Giese G, Wunderlich F (1998) Estradiol binding to cell surface raises cytosolic free calcium in T cells. FEBS Lett 422:349–353

Benten WPM, Lieberherr M, Giese G, Wrehlke C, Stamm O, Sekeris CE, Mossmann H, Wunderlich F (1999) Functional testosterone receptors in plasma membranes of T cells. FASEB J 13:123–133

Bernin H, Lotter H (2014) Sex bias in the outcome of human tropical infectious diseases: influence of steroid hormones. J Infect Dis 209(Suppl 3):S107–S113. doi:10.1093/infdis/jit610

Beverley JK, Fleck DG, Kwantes W, Ludlam GB (1976) Age-sex distribution of various diseases with particular reference to toxoplasmic lymphadenopathy. J Hyg 76:215–228

Blessmann J, Van Linh P, Nu PA, Thi HD, Muller-Myhsok B, Buss H, Tannich E (2002) Epidemiology of amebiasis in a region of high incidence of amebic liver abscess in central Vietnam. Am J Trop Med Hyg 66:578–583

Boyer K, McLeod R (1998) Toxoplasmosis, vol 286, 1st edn. Churchill Livingstone, New York

Boyer KM, Holfels E, Roizen N, Swisher C, Mack D, Remington J, Withers S, Meier P, McLeod R (2005) Toxoplasmosis Study Group. Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital toxoplasmosis: implications for prenatal management and screening. Am J Obstet Gynecol 192:564–571

Bryson KJ, Millington OR, Mokgethi T, McGachy HA, Brombacher F, Alexander J (2001) BALB/c mice deficient in CD4 T cell IL-4Rα expression control *Leishmania mexicana* Load although female but not male mice develop a healer phenotype. PLoS Negl Trop Dis 5(1):e930

Carlier Y, Truyens C, Deloron P, Peyron F (2012) Congenital parasitic infections: a review. Acta Trop 121:55–70

Cattell RB, Cattell AK, Cattell HEP (1993) 16PF fifth edition questionnaire. Institute for Personality and Ability Testing, Champaign, IL

Chesnais CB, Missamou F, Pion SD, Bopda J, Louya F, Majewski AC, Fischer PU, Weil GJ, Boussinesq M (2014) A case study of risk factors for lymphatic filariasis in the Republic of Congo. Parasit Vectors 7:300. doi:10.1186/1756-3305-7-300

Chiang TY, Hsieh HH, Kuo MC, Chiu KT, Lin WC, Fan CK, Fang CT, Ji DD (2012) Seroepidemiology of Toxoplasma gondii Infection among healthy blood donors in Taiwan. PLoS ONE 7:e48139

Conceição-Silva F, Morgado FN, Pimentel MI, e Vasconcellos E d C, Schubach AO, Valete-Rosalino CM, Kropf P, Müller I (2013) Two women presenting worsening cutaneous ulcers during pregnancy: diagnosis, immune response, and follow-up. PLoS Negl Trop Dis 7:e2472

Cucunubá ZM, Guerra Á, Rivera JA, Nicholls RS (2013) Comparison of asymptomatic *Plasmodium* spp. infection in two malaria-endemic Colombian locations. Trans R Soc Trop Med Hyg 107(2):129–136

Daryani A, Sarvi S, Aarabi M, Mizani A, Ahmadpour E, Shokri A, Rahimi MT, Sharif M (2014) Seroprevalence of Toxoplasma gondii in the Iranian general population: a systematic review and meta-analysis. Acta Trop 137:185–194

Dauby N, Alonso-Vega C, Suarez E, Flores A, Hermann E, Córdova M, Tellez T, Torrico F, Truyens C, Carlier Y (2009) Maternal infection with *Trypanosoma cruzi* and congenital Chagas disease induce a trend to a type 1 polarization of infant immune responses to vaccines. PLoS Negl Trop Dis 3(12):e571

Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, Newman RD (2007) Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis 7:93–104

Druckmann R, Druckmann MA (2005) Progesterone and the immunology of pregnancy. J Steroid Biochem Mol Biol 97:389–396

Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, Hutchinson P, Steketee RW (2012) Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. Lancet Infect Dis 12:942–949

Elias D, Britton S, Aseffa A, Engers H, Akuffo H (2008) Poor immunogenicity of BCG in helminth infected population is associated with increased in vitro TGF-beta production. Vaccine 26:3897–3902

Eloi-Santos SM, Novato-Silva E, Maselli VM, Gazzinelli G, Colley DG, Correa-Oliveira R (1989) Idiotypic sensitization in utero of children born to mothers with schistosomiasis or Chagas' disease. J Clin Invest 84(3):1028–1031

Fairley JK, Bisanzio D, King CH, Kitron U, Mungai P, Muchiri E, King CL, Malhotra I (2013) Birthweight in offspring of mothers with high prevalence of helminth and malaria infection in coastal Kenya. Am J Trop Med Hyg 88:48–53. doi:10.4269/ajtmh. 2012.12-0371

Fallon PG, Mangan NE (2007) Suppression of TH2-type allergic reactions by helminth infection. Nat Rev Immunol 7:220–230

Felix JP, Lira RP, Zacchia RS, Toribio JM, Nascimento MA, Arieta CE (2014) Trimethoprim-sulfamethoxazole versus placebo to reduce the risk of recurrences of *Toxoplasma gondii* retinochoroiditis: randomized controlled clinical trial. Am J Ophthalmol 157:762–766.e1

Figueiredo CA, Barreto ML, Rodrigues LC, Cooper PJ, Silva NB, Amorim LD, Alcantara-Neves NM (2010) Chronic intestinal helminth infections are associated with immune hyporesponsiveness and induction of a regulatory network. Infect Immun 78:3160–3167

Flegr J (2007) Effects of toxoplasma on human behavior. Schizophr Bull 33:757–760

Flegr J, Preiss M, Klose J, Havlícek J, Vitáková M, Kodym P (2003) Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite *Toxoplasma gondii* dopamine, a missing link between schizophrenia and toxoplasmosis? Biol Psychol 63:253–268

Flegr J, Lenochová P, Hodný Z, Vondrová M (2011) Fatal attraction phenomenon in humans: cat odour attractiveness increased for toxoplasma-infected men while decreased for infected women. PLoS Negl Trop Dis 5:e1389

Franco PS, Ribeiro M, Lopes-Maria JB, Costa LF, Silva DA, de Freitas BB, de Oliveira GA, Mineo JR, Ferro EA (2014) Experimental infection of Calomys callosus with atypical strains of Toxoplasma gondii shows gender differences in severity of infection. Parasitol Res 113(7):2655–2664

Ganley L, Rajan TV (2001) Endogenous testosterone levels do not affect filarial worm burdens in mice. Exp Parasitol 98:29–34

Garweg JG, Scherrer J, Wallon M, Kodjikian L, Peyron F (2005) Reactivation of ocular toxoplasmosis during pregnancy. BJOG 112:241–242

Gatkowska J, Wieczorek M, Dziadek B, Dzitko K, Dlugonska H (2013) Sex-dependent neurotransmitter level changes in brains of *Toxoplasma gondii* infected mice. Exp Parasitol 133:1–7

Gay-Andrieu F, Cozon GJ, Ferrandiz J, Peyron F (2002) Progesterone fails to modulate Toxoplasma gondii replication in the RAW 264.7 murine macrophage cell line. Parasite Immunol 24:173–178

Grainger JR, Smith KA, Hewitson JP, McSorley HJ, Harcus Y, Filbey KJ, Finney CA, Greenwood EJ, Knox DP, Wilson MS, Belkaid Y, Rudensky AY, Maizels RM (2010) Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-β pathway. J Exp Med 207:2331–2341. doi:10.1084/jem.20101074

Guadalupe I, Mitre E, Benitez S, Chico ME, Nutman TB, Cooper PJ (2009) Evidence for in utero sensitization to *Ascaris lumbricoides* in newborns of mothers with ascariasis. J Infect Dis 199:1846–1850

Gulgun M, Balci E, Karaoğlu A, Babacan O, Türker T (2013) Pediculosis capitis: prevalence and its associated factors in primary school children living in rural and urban areas in Kayseri, Turkey. Cent Eur J Public Health 21:104–108

Haider BA, Humayun Q, Bhutta ZA (2009) Effect of administration of antihelminthics for soil transmitted helminths during pregnancy. Cochrane Database Syst Rev. doi:10.1002/14651858.CD005547.pub2

Haque U, Sunahara T, Hashizume M, Shields T, Yamamoto T, Haque R, Glass GE (2011) Malaria prevalence, risk factors and spatial distribution in a hilly forest area of Bangladesh. PLoS ONE 6:e18908

Hara T, Ohashi S, Yamashita Y, Abe T, Hisaeda H, Himeno K, Good RA, Takeshita K (1996) Human V delta 2+ gamma delta T-cell tolerance to foreign antigens of *Toxoplasma gondii*. Proc Natl Acad Sci USA 93:5136–5140

Harris NL, Spoerri I, Schopfer JF, Nembrini C, Merky P, Massacand J, Urban JF Jr, Lamarre A, Burki K, Odermatt B, Zinkernagel RM, Macpherson AJ (2006) Mechanisms of neonatal mucosal antibody protection. J Immunol 177:6256–6262

Hayes KS, Hager R, Grencis RK (2014) Sex-dependent genetic effects on immune responses to a parasitic nematode. BMC Genomics 15:193

Helk E, Bernin H, Ernst T, Ittrich H, Jacobs T, Heeren J, Tacke F, Tannich E, Lotter H (2013) TNFα-mediated liver destruction by Kupffer cells and Ly6Chi monocytes during Entamoeba histolytica infection. PLoS Pathog 9(1):e1003096. doi:10.1371/journal.ppat.1003096

Henriquez SA, Brett R, Alexander J, Pratt J, Roberts CW (2009) Neuropsychiatric disease and *Toxoplasma gondii* infection. Neuroimmunomodulation 16:123–133

Hepworth MR, Grencis RK (2009) Disruption of Th2 immunity results in a gender-specific expansion of IL-13 producing accessory NK cells during helminth infection. J Immunol 183:3906–3914

Hepworth MR, Hardman MJ, Grencis RK (2010) The role of sex hormones in the development of Th2 immunity in a gender-biased model of *Trichuris muris* infection. Eur J Immunol 40:406–416

Hernández-Bello R, Ramirez-Nieto R, Muñiz-Hernández S, Nava-Castro K, Pavón L, Sánchez-Acosta AG, Morales-Montor J (2011) Sex steroids effects on the molting process of the helminth human parasite *Trichinella spiralis*. J Biomed Biotechnol 2011:625380. doi:10.1155/2011/625380

Hewitson JP, Grainger JR, Maizels RM (2009) Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. Mol Biochem Parasitol. doi:10.1016/j.molbiopara.2009.04.008

Hodková H, Kolbeková P, Skallová A, Lindová J, Flegr J (2007) Higher perceived dominance in *Toxoplasma* infected men – A new evidence for role of increased level of testosterone in toxoplasmosis-associated changes in human behavior. Neuro Endocrinol Lett 28:110–114

Hotez PJ, Kamath A (2009) Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. PLoS Negl Trop Dis. doi:10.1371/journal.pntd.0000412

Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J (2008) Helminth infections: the great neglected tropical diseases. J Clin Invest 118:1311–1321. doi:10.1172/JCI34261

Huerta L, Terrazas LI, Sciutto E, Larralde C (1992) Immunological mediation of gonadal effects on experimental murine cysticercosis caused by *Taenia crassiceps* metacestodes. J Parasitol 78:471–476

Huldt G, Lagercrantz R, Sheehe PR (1979) On the epidemiology of human toxoplasmosis in Scandinavia especially in children. Acta Paediatr Scand 68(5):745–749

Jones JL, Roberts JM (2012) Toxoplasmosis hospitalizations in the United States, 2008, and trends, 1993–2008. Clin Infect Dis 254:e58–e61

Jones LA, Anthony JP, Henriquez FL, Lyons RE, Nickdel MB, Carter KC, Alexander J, Roberts CW (2008) Toll-like receptor-4-mediated macrophage activation is differentially regulated by progesterone via the glucocorticoid and progesterone receptors. Immunology 125:59–69

King CL, MalhotraI MP, Wamachi A, Kioko J, Ouma JH, Kazura JW (1998) B cell sensitization to helminthic infection develops in utero in humans. J Immunol (Baltimore, MD: 1950) 160 (7):3578–3584

Kittas C, Henry L (1979a) Effect of gonadectomy and oestrogen administration on the response of lymph-node post-capillary venules to infection with Toxoplasma gondii. J Pathol 127:129–136

Kittas C, Henry L (1979b) Effect of sex hormones on the immune system of guinea-pigs and on the development of toxoplasmic lesions in non-lymphoid organs. Clin Exp Immunol 36:16–23

Kittas C, Henry L (1980) Effect of sex hormones on the response of mice to infection with *Toxoplasma gondii*. Br J Exp Pathol 61:590–600

Kittas S, Kittas C, Paizi-Biza P, Henry L (1984) A histological and immunohistochemical study of the changes induced in the brains of white mice by infection with *Toxoplasma gondii*. Br J Exp Pathol 65:67–74

Klein SL (2004) Hormonal and immunological mechanisms mediating sex differences in parasite infection. Parasite Immunol 26:247–264

Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, Hellings JA, Toovey S, Prinssen EP (2014) Maternal immune activation and abnormal brain development across CNS disorders. Nat Rev Neurol. doi:10.1038/nrneurol.2014.187

Kochar DK, Thanvi I, Joshi A, Shubhakaran, Agarwal N, Jain N (1999) Mortality trends in falciparum malaria–effect of gender difference and pregnancy. J Assoc Physicians India 47(8):774–778

Kodjikian L, Hoigne I, Adam O, Jacquier P, Aebi-Ochsner C, Aebi C, Garweg JG (2004) Vertical transmission of toxoplasmosis from a chronically infected immunocompetent woman. Pediatr Infect Dis J 23(3):272–274

Krishnan L, Guilbert LJ, Russell AS, Wegmann TG, Mosmann TR, Belosevic M (1996a) Pregnancy impairs resistance of C57BL/6 mice to *Leishmania major* infection and causes decreased antigen-specific IFN-gamma response and increased production of T helper 2 cytokines. J Immunol 156:644–652

Krishnan L, Guilbert LJ, Wegmann TG, Belosevic M, Mosmann TR (1996b) T helper 1 response against Leishmania major in pregnant C57BL/6 mice increases implantation failure and fetal resorptions: correlation with increased IFN-gamma and TNF and reduced IL-10 production by placental cells. J Immunol 156:653–662

Krucken J, SchmittWrede HP, MarkmannMulisch U, Wunderlich F (1997) Novel gene expressed in spleen cells mediating acquired testosterone-resistant immunity to *Plasmodium chabaudi* malaria. Biochem Biophys Res Commun 230:167–170

Krucken J, Stamm O, Schmitt-Wrede H-P, Mincheva A, Lichter P, Wunderlich F (1999) Spleen-specific expression of the malaria-inducible intronless mouse gene imap38. J Biol Chem 274:24383–24391

Kuris AM (2012) The global burden of human parasites: who and where are they? How are they transmitted? J Parasitol 98:1056–1064

Kurtis JD, Higashi A, Wu HW, Gundogan F, McDonald EA, Sharma S, PondTor S, Jarilla B, Sagliba MJ, Gonzal A, Olveda R, Acosta L, Friedman JF (2011) Maternal Schistosomiasis japonica is associated with maternal, placental, and fetal inflammation. Infect Immun 79:1254–1261. doi:10.1128/IAI. 01072-10

Labeaud AD, Malhotra I, King MJ, King CL, King CH (2009) Do antenatal parasite infections devalue childhood vaccination? PLoS Negl Trop Dis 3(5):e442

Larocque R, Casapia M, Gotuzzo E, MacLean JD, Soto JC, Rahme E, Gyorkos TW (2006) A double-blind randomized controlled trial of antenatal mebendazole to reduce low birthweight in a hookworm-endemic area of Peru. Trop Med Int Health 11:1485–1495

Larralde C, Morales J, Terrazas I, Govezensky T, Romano MC (1995) Sex hormone changes induced by the parasite lead to feminization of the male host in murine *Taenia crassiceps* cysticercosis. J Steroid Biochem Mol Biol 52:575–580

Liesenfeld O, Nguyen TA, Pharke C, Suzuki Y (2001) Importance of gender and sex hormones in regulation of susceptibility of the small intestine to peroral infection with *Toxoplasma gondii* tissue cysts. J Parasitol 87:1491–1493

Lin H, Lu L, Tian L, Zhou S, Wu H, Bi Y, Ho SC, Liu Q (2009) Spatial and temporal distribution of falciparum malaria in China. Malar J 8:130

Lotter H, Helk E, Bernin H, Jacobs T, Prehn C, Adamski J, González-Roldán N, Holst O, Tannich E (2013) Testosterone increases susceptibility to amebic liver abscess in mice and mediates inhibition of IFNγ secretion in natural killer T cells. PLoS ONE 8(2):e55694. doi:10.1371/journal.pone.0055694

Lynch NR, Yazbel L, Verde O, Avila JL, Monzon H, Convit J (1982) Delayed-type hypersensitivity and immunoglobulin E in American cutaneous leishmaniasis. Infect Immun 38:877–880

Machado-Coelho GL, Caiaffa WT, Genaro O, Magalhães PA, Mayrink W (2005) Risk factors for mucosal manifestation of American cutaneous leishmaniasis. Trans R Soc Trop Med Hyg 99:55–61

Malhotra I, Mungai P, Wamachi A, Kioko J, Ouma JH, Kazura JW, King CL (1999) Helminth- and Bacillus Calmette-Guérin-induced immunity in children sensitized in utero to filariasis and schistosomiasis. J Immunol (Baltimore, MD: 1950) 162(11):6843–6848

McClure EM, Meshnick SR, Mungai P, Malhotra I, King CL, Goldenberg RL, Hudgens MG, Siega-Riz AM, Dent AE (2014) The association of parasitic infections in pregnancy and

maternal and fetal anemia: a cohort study in coastal Kenya. PLoS Negl Trop Dis 8(2):e2724. doi:10.1371/journal.pntd.0002724

McDonald EA, Friedman JF, Sharma S, Acosta L, Pond-Tor S, Cheng L, White ES, Kurtis JD (2013) *Schistosoma japonicum* soluble egg antigens attenuate invasion in a first trimester human placental trophoblast model. PLoS Negl Trop Dis 7(6):e2253. doi:10.1371/journal.pntd.0002253

McDonald EA, Cheng L, Jarilla B, Sagliba MJ, Gonzal A, Amoylen AJ, Olveda R, Acosta L, Baylink D, White ES, Friedman JF, Kurtis JD (2014) Maternal infection with Schistosoma japonicum induces a profibrotic response in neonates. Infect Immun 82(1):350–355. doi:10.1128/IAI. 01060-13

McLeod R, Beem MO, Estes RG (1985) Lymphocyte anergy specific to Toxoplasma gondii antigens in a baby with congenital toxoplasmosis. J Clin Lab Immunol 17:149e153

McLeod R, Mack DG, Boyer K, Mets M, Roizen N, Swisher C, Patel D, Beckmann E, Vitullo D, Johnson D et al (1990) Phenotypes and functions of lymphocytes in congenital toxoplasmosis. J Lab Clin Med 116:623–635

McMahon-Pratt D, Alexander J (2004) Does the *Leishmania major* paradigm of pathogenesis and protection hold for New World cutaneous leishmaniases or the visceral disease? Immunol Rev 201:206–224

McSorley HJ, Maizels RM (2012) Helminth infections and host immune regulation. Clin Microbiol Rev 25:585–608. doi:10.1128/CMR. 05040-11

Mehta RS, Rodriguez A, Chico M, Guadalupe I, Broncano N, Sandoval C, Tupiza F, Mitre E, Cooper PJ (2012) Maternal geohelminth infections are associated with an increased susceptibility to geohelminth infection in children: a case–control study. PLoS Negl Trop Dis 6(7): e1753. doi:10.1371/journal.pntd.0001753

Menendez C (1995) Malaria during pregnancy: a priority area of malaria research and control. Parasitol Today 5:178–183

Mizgajska-Wiktor H, Jarosz W, Andrzejewska I, Krzykała M, Janowski J, Kozłowska M (2013) Differences in some developmental features between *Toxoplasma gondii*-seropositive and seronegative school children. Folia Parasitol (Praha) 60:416–424

Mock BA, Nacy CA (1988) Hormonal modulation of sex differences in resistance to *Leishmania major* systemic infections. Infect Immun 56:3316–3319

Montesano MA, Colley DG, Eloi-Santos S, Freeman GL, Secor WE (1999a) Neonatal idiotypic exposure alters subsequent cytokine, pathology, and survival patterns in experimental Schistosoma mansoni infections. J Exp Med 189:637–645

Montesano MA, Colley DG, Freeman GL, Secor WE (1999b) Neonatal exposure to idiotype induces Schistosoma mansoni egg antigen-specific cellular and humoral immune responses. J Immunol (Baltimore, MD: 1950) 163:898–905

Morales-Montor J, Chavarria A, De León MA, Del Castillo LI, Escobedo EG, Sánchez EN, Vargas JA, Hernández-Flores M, Romo-González T, Larralde C (2004) Host gender in parasitic infections of mammals: an evaluation of the female host supremacy paradigm. J Parasitol 90(3):531–546

Morgan DJ, Guimaraes LH, Machado PR, D'Oliveira A Jr, Almeida RP, Lago EL, Faria DR, Tafuri WL, Dutra WO, Carvalho EM (2007) Cutaneous leishmaniasis during pregnancy: exuberant lesions and potential fetal complications. Clin Infect Dis 45:478–482

Mpairwe H, Webb EL, Muhangi L, Ndibazza J, Akishule D, Nampijja M, Ngom-wegi S, Tumusime J, Jones FM, Fitzsimmons C, Dunne DW, Muwanga M, Rodrigues LC, Elliott AM (2011) Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. Pediatr Allergy Immunol 22:305–312. doi:10.1111/j.1399-3038.2010.01122.x

Mwakitalu ME, Malecela MN, Mosha FW, Simonsen PE (2014) Urban schistosomiasis and soil transmitted helminthiases in young school children in Dar es Salaam and Tanga, Tanzania, after a decade of anthelminthic intervention. Acta Trop 133:35–41. doi:10.1016/j.actatropica.2014.01.012

Nakazawa M, Fantappie MR, Freeman GL Jr, Eloi-Santos S, Olsen NJ, Kovacs WJ, Secor WE, Colley DG (1997) Schistosoma mansoni: susceptibility differences between male and female mice can be mediated by testosterone during early infection. Exp Parasitol 85:233–240

Nava-Castro K, Hernández-Bello R, Muñiz-Hernández S, Camacho-Arroyo I, Morales-Montor J (2012) Sex steroids, immune system, and parasitic infections: facts and hypotheses. Ann NY Acad Sci 1262:16–26. doi:10.1111/j.1749-6632.2012.06632.x

Ndibazza J, Mpairwe H, Webb EL, Mawa PA, Nampijja M, Muhangi L, Kihembo M, Lule SA, Rutebarika D, Apule B, Akello F, Akurut H, Oduru G, Naniima P, Kizito D, Kizza M, Kizindo R, Tweyongere R, Alcock KJ, Muwanga M, Elliott AM (2012) Impact of anthelminthic treatment in pregnancy and childhood on immunisations, infections and eczema in childhood: a randomised controlled trial. PLoS ONE 7(12):e50325. doi:10.1371/journal.pone.0050325

Novotná M, Hanusova J, Klose J, Preiss M, Havlicek J, Roubalová K, Flegr J (2005) Probable neuroimmunological link between *Toxoplasma* and cytomegalovirus infections and personality changes in the human host. BMC Infect Dis 5:54

Pathak S, Rege M, Gogtay NJ, Aigal U, Sharma SK, Valecha N, Bhanot G, Kshirsagar NA, Sharma S (2012) Age-dependent sex bias in clinical malarial disease in hypoendemic regions. PLoS ONE 7:e35592

Pfaff AW, de-la-Torre A, Rochet E, Brunet J, Sabou M, Sauer A, Bourcier T, Gomez-Marin JE, Candolfi E (2014) New clinical and experimental insights into Old World and neotropical ocular toxoplasmosis. Int J Parasitol 44:99–107

Phillips AN, Antunes F, Stergious G, Ranki A, Jensen GF, Bentwich Z, Sacks T, Pedersen C, Lundgren JD, Johnson AM (1994) A sex comparison of rates of new AIDS-defining disease and death in 2554 AIDS cases. AIDS in Europe Study Group. AIDS 8:831–835

Pinot de Moira A, Fulford AJ, Kabatereine NB, Ouma JH, Booth M, Dunne DW (2010) Analysis of complex patterns of human exposure and immunity to Schistosomiasis mansoni: the influence of age, sex, ethnicity and IgE. PLoS Negl Trop Dis 4(9):e820. doi:10.1371/journal.pntd.0000820

Pullan RL, Bethony JM, Geiger SM, Cundill B, Correa-Oliveira R, Quinnell RJ, Brooker S (2008) Human helminth co-infection: analysis of spatial patterns and risk factors in a Brazilian community. PLoS Negl Trop Dis 2(12):e352. doi:10.1371/journal.pntd.0000352

Rajan TV, Bailis JM, Yates JA, Shultz LD, Greiner DL, Nelson FK (1994a) Maternal influence on susceptibility of offspring to *Brugia malayi* infection in a murine model of filariasis. Acta Trop 58:283–289

Rajan TV, Nelson FK, Shultz LD, Shultz KL, Beamer WG, Yates J, Greiner DL (1994b) Influence of gonadal steroids on susceptibility to *Brugia malayi* in scid mice. Acta Trop 56:307–314

Roberts CW, Alexander J (1992) Studies on a murine model of congenital toxoplasmosis: vertical disease transmission only occurs in BALB/c mice infected for the first time during pregnancy. Parasitology 104:19–23

Roberts CW, Cruickshank SM, Alexander J (1995) Sex-determined resistance to *Toxoplasma gondii* is associated with temporal differences in cytokine production. Infect Immun 63:2549–2555

Roberts CW, Satoskar A, Alexander J (1996) Sex steroids, pregnancy-associated hormones and immunity to parasitic infection. Parasitol Today 12:382–388

Roberts CW, Walker W, Alexander J (2001) Sex-associated hormones and immunity to protozoan parasites. Clin Microbiol Rev 14(3):476–88

Roberts C, Prasad S, Khaliq F, Gazzinelli R, Khan I, McLeod R (2014) Adaptive immunity and genetics of the host immune response. In: Weiss LM, Kim K (eds) *Toxoplasma gondii*: the model apicomplexan perspectives and methods, 2nd edn. London, pp 819–994

Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW (2007) Malaria in pregnancy: pathogenesis and immunity. Lancet Infect Dis 7:105–117

Saito S, Nakashima A, Shima T, Ito M (2010) Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol 63:601–610. doi:10.1111/j.1600-0897.2010.00852.x

Saito S, Nakashima A, Ito M, Shima T (2011) Clinical implication of recent advances in our understanding of IL-17 and reproductive immunology. Expert Rev Clin Immunol 7:649–657. doi:10.1586/eci.11.49

Satoskar A, Alexander J (1995) Sex-determined susceptibility and differential IFN-gamma and TNF-alpha mRNA expression in DBA/2 mice infected with *Leishmania mexicana*. Immunology 84:1–4

Satoskar A, Al-Quassi HH, Alexander J (1998) Sex-determined resistance against *Leishmania mexicana* is associated with the preferential induction of a Th1-like response and IFN-gamma production by female but not male DBA/2 mice. Immunol Cell Biol 76:159–166

Shirahata T, Muroya N, Ohta C, Goto H, Nakane A (1992) Correlation between increased susceptibility to primary Toxoplasma gondii infection and depressed production of gamma interferon in pregnant mice. Microbiol Immunol 36:81–91

Silveira C, Ferreira R, Muccioli C, Nussenblatt R, Belfort R Jr (2003) Toxoplasmosis transmitted to a newborn from the mother infected 20 years earlier. Am J Ophthalmol 136(2):370–371

Skallová A, Novotná M, Kolbeková P, Gasová Z, Veselý V, Sechovská M, Flegr J (2005) Decreased level of novelty seeking in blood donors infected with *Toxoplasma*. Neuro Endocrinol Lett 26:480–486

Soares L, Abad-Franch F, Ferraz G (2014) Epidemiology of cutaneous leishmaniasis in central Amazonia: a comparison of sex-biased incidence among rural settlers and field biologists. Trop Med Int Health 19:988–995

Steketee RW (2014) Malaria prevention during pregnancy-is there a next step forward? PLoS Med 11:e1001734

Steketee RW, Nahlen BL, Parise ME, Menendez C (2001) The burden of malaria in pregnancy in malaria-endemic areas. Am J Trop Med Hyg 64(1–2 Suppl):28–35

Tareen AM, Rafique M, Wadood A, Qasim M, Rahman H, Shah SH, Khan K, Pirkani GS (2012) Malaria burden in human population of Quetta, Pakistan. Eur J Microbiol Immunol (Bp) 2:201–204

Togno-Peirce C, Nava-Castro K, Terrazas LI (2013) Morales-Montor J (2013) Sex-associated expression of co-stimulatory molecules CD80, CD86, and accessory molecules, PDL-1, PDL-2 and MHC-II, in F480+ macrophages during murine cysticercosis. Biomed Res Int 2013:570158. doi:10.1155/2013/570158

Torgerson PR, Mastroiacovo P (2013) The global burden of congenital toxoplasmosis: a systematic review. Bull World Health Organ 91:501–508

Torrey EF, Yolken RH (2013) *Toxoplasma* oocysts as a public health problem. Trends Parasitol 29:380–384. doi:10.1016/j.pt.2013.06.001

van Riet E, Hartgers FC, Yazdanbakhsh M (2007) Chronic helminth infections induce immunomodulation: consequences and mechanisms. Immunobiology 212(6):475–490

Venugopalan PP, Shenoy DU, Kamath A, Rajeev A (1997) Distribution of malarial parasites: effect of gender of construction workers. Indian J Med Sci 51(3):89–92

Vercruysse J, Behnke JM, Albonico M, Ame SM, Angebault C, Bethony JM, Engels D, Guillard B, Nguyen TV, Kang G, Kattula D, Kotze AC, McCarthy JS, Mekonnen Z, Montresor A, Periago MV, Sumo L, Tchuenté LA, Dang TC, Zeynudin A, Levecke B (2011) Assessment of the anthelmintic efficacy of albendazole in school children in seven countries where soil-transmitted helminths are endemic. PLoS Negl Trop Dis 5:e948. doi:10.1371/journal.pntd.0000948

Vogel N, Kirisits M, Michael E, Bach H, Hostetter M, Boyer K, Simpson R, Holfels E, Hopkins J, Mack D, Mets MB, Swisher CN, Patel D, Roizen N, Stein L, Stein M, Withers S, Mui E, Egwuagu C, Remington J, Dorfman R, McLeod R (1996) Congenital toxoplasmosis transmitted from an immunologically competent mother infected before conception. Clin Infect Dis 23(5):1055–1060

Webb EL, Mawa PA, Ndibazza J, Kizito D, Namatovu A, Kyosiimire-Lugemwa J, Nanteza B, Nampijja M, Muhangi L, Woodburn PW, Akurut H, Mpairwe H, Akello M, Lyadda N, Bukusuba J, Kihembo M, Kizza M, Kizindo R, Nabulime J, Ameke C, Namujju PB,

Tweyongyere R, Muwanga M, Whitworth JA, Elliott AM (2011) Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. Lancet 377(9759):52–62

Weil GJ, Hussain R, Kumaraswami V, Tripathy SP, Phillips KS, Ottesen EA (1983) Prenatal allergic sensitization to helminth antigens in offspring of parasite-infected mothers. J Clin Invest 71(5):1124–1129

Xiao J, Kannan G, Jones-Brando L, Brannock C, Krasnova IN, Cadet JL, Pletnikov M, Yolken RH (2012) Sex-specific changes in gene expression and behavior induced by chronic Toxoplasma infection in mice. Neuroscience 206:39–48

Yolken RH, Dickerson FB, Fuller TE (2009) Toxoplasma and schizophrenia. Parasite Immunol 31(11):706–715

Chapter 14
Epilogue: Future of Sex and Gender-Based Studies in Infectious Diseases

Sabra L. Klein and Craig W. Roberts

The topic of male–female differences in disease outcomes continues to receive attention in both the scientific literature and lay press. There is little debate about whether the sexes are behaviorally and biologically different, but how this impacts disease processes and the pipeline for developing drugs, vaccines, medical devices, and policy decisions is highly debated. The current book as well as a previous book (Klein and Roberts 2010) that we published in 2010 illustrates that the sexes differ in their exposure, immune responses, and outcome of diverse infectious diseases and inflammatory conditions. While the intensity and prevalence of infections are often higher for males, the outcome of diseases, including those caused by HIV, influenza, hemorrhagic fever viruses, *Toxoplasma gondii*, and *Borrelia burgdorferi* to name a few, can be worse for females. As detailed in Chapter 1 of this book, females tend to mount higher innate and adaptive immune responses, which can result in faster clearance of pathogens, but also may contribute to increased development of immunopathology and inflammatory conditions. Responses to prophylaxis and therapeutic treatments for infectious diseases also differ between the sexes, with females typically experiencing greater adverse reactions than males (Chapter 4). These sex differences can vary by age and reproductive status (Chapters 3 and 10), illustrating that these differences are not fixed, but are variable across the life course. Despite sex being the most evolutionarily well conserved and easily disaggregated variable by which to compare the outcome of diseases and their treatments, it is often ignored in the biomedical sciences. The challenges of

S.L. Klein (✉)
Department of Molecular Microbiology & Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MA, USA
e-mail: sklein2@jhu.edu

C.W. Roberts
Professor of Parasitology, Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, United Kingdom
e-mail: c.w.roberts@strath.ac.uk

S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0_14

including women in clinical trials are in some cases obvious and include the potential of hormonal variations during menstrual cycles and their cessation at menopause. These factors are further complicated due to pregnancy (when hormone levels change and the fetus could be at risk during a trial) or artificial administration of hormones as contraceptives or for hormone replacement therapy. However, the scientific, medical, and ethical cases for including males and females in preclinical and clinical trials are too profound to ignore.

14.1 Regulators Push for Disaggregation of Data by Sex or Gender

In an effort to protect vulnerable populations from adverse drug and treatment effects, the Food and Drug Administration (FDA) in the United States published guidelines in 1977 advising that women of childbearing potential be excluded from drug development studies (Guidelines 1977), which was interpreted to mean that women should be excluded from most clinical trials. Similar policies and practices have excluded women from clinical trials in other geographical regions. Over time, this resulted in inadequate representation of women in many clinical trials, and in the 1990s, both the FDA and the National Institutes of Health (NIH) began recommending that clinical trials include women as subjects (Parekh et al. 2011; Pinn 1994). Although women are now included in clinical trials for drugs and vaccines, there is still inadequate analysis of outcome data by sex (Beery and Zucker 2011). It is often not considered whether adverse reactions, dosages, or the efficacy of drugs or vaccines are different between the sexes. Yet in an analysis of drugs withdrawn from the US market in 2005, it was reported that 8 out of every 10 drugs were taken off the market because of greater adverse side effects in women compared with men (Simon 2005). Almost 20 years after requesting inclusion of women in clinical trials, it was recognized that most preclinical biomedical research has been conducted with inadequate consideration of sex (Yoon et al. 2014). Male sex bias is most extreme in pharmacology, where as female sex bias is most pervasive in immunology and infectious diseases (Beery and Zucker 2011). In response to the prevalent biases in preclinical research, NIH announced plans to require sex inclusion in preclinical research using animal or primary cell culture models (Clayton and Collins 2014). While Canadian funding agencies led the way and have been requesting sex inclusion in preclinical and clinical studies, European, South American, and Asian funding agencies lag behind with no clear instructions for investigators about sex inclusion. We hope that collaborative projects, such as the current volume, will raise awareness and provide necessary evidence to inform policy decisions in other countries.

14.2 Studying Sex Differences Saves Lives and Money

In 2013, the United States FDA recommended lower doses of insomnia drugs for women than men (FDA 2013). Drugs containing zolpidem, in particular, remain in circulation longer, take longer to metabolize, and result in a longer duration of impairment of mental alertness in women compared with men. The FDA cut the recommended dose of sleep medications that contain zolpidem in half for women (FDA 2013). Media outlets extensively reported this story as novel and newsworthy—primarily on the basis that drugs may need to be administered with sex-specific dosage recommendations. This is however the very basis of personalized medicine. While personalized medicine has begun to be applied to drugs (see Chapter 4), more novel has been its application to biologics such as vaccines. To date, there are no examples of sex-specific doses of vaccines. As one example, which is discussed in Chapter 11, in clinical trials of a herpes simplex virus (HSV) vaccine, no overall protection from infection was observed in phase 1 or 2 trials (Stanberry et al. 2002). When data were analyzed by sex, the efficacy of the vaccine was 73 % in women and only 11 % in men, indicating that the vaccine was able to provide protection against development of symptoms associated with genital herpes in women, but not in men. There currently is no approved HSV vaccine. Harnessing these clinical observations and combining this with extensive, rigorously controlled preclinical studies in animals and cells will be necessary to demonstrate that consideration of the variable "sex" could save both lives and money and contribute to better and more efficacious treatments for infectious diseases.

14.3 Limitations of Current Knowledge

As we experience broader acceptance of the effects of sex on disease pathogenesis, prevention, and treatment, it will be important to identify and address the limitations of our knowledge. For example, it will require considerable work to translate empirical observations of sex differences into studies of the proximal mechanisms responsible. This might allow these mechanisms to be mitigated, augmented, as appropriate. Logically, sex differences can derive from sex chromosomes and thus genes, hormone levels (i.e., sex steroids and others), or physiological differences (i.e., percentage and distribution of body fat and muscle, relative size of liver, blood volume, or relative expression of enzymes). Deconvolution of some of these factors might prove a challenge as many of these parameters vary within one sex, with other parameters showing some overlap between the sexes. Gender differences, such as behavior and social circumstances that vary between societies and therefore study populations, need to be taken into account as well. Any of the above parameters could have important secondary effects on males and females, such as influencing the microbiome (see Chapter 1).

The role of the gut microbiome in human health and disease has been increasingly acknowledged and differences between the microbiomes of male and female fish, mice, and humans are now known to exist (Bolnick et al., 2014), suggesting that these differences are evolutionarily well conserved. Differences in the vaginal microbiome are known to exist between women, and although the microbiome would appear to be relatively stable in humans throughout the menstrual cycle, changes are evident following the menopause (Chaban et al. 2014). These data suggest that there may be long-term but not short-term hormonal influences on the microbiomes of humans. These data provide a conceptual route from hormonal status to microbiome to disease, but establishing this experimentally will be challenging.

14.4 Recommendations

Moving forward, the scientific community should address previous shortcomings in experimental design and reporting of sex-based differences. A recent report which examined the quality of sex-specific reporting in animal trypanosomiasis experiments found that despite known sex differences in mice with trypanosomiasis, only 70 % of the studies reported the sex of animals used in the experimental infection, and fewer (25 %) of the studies reported the sex of animals used to maintain their parasite stocks (Flórez-Vargas et al., 2014). Similar omissions in other diseases are evident in the literature (Beery and Zucker 2011). While regulatory agencies are tightening the rules that govern clinical trials and preclinical research studies, scientific publishers and reviewers should be educated about sex differences to increase the quality of reporting of animal and cell-based experiments to include sex.

14.5 Conclusions

Our goal in editing this book was to consolidate the literature on sex differences in infection and treatments for infectious diseases to illustrate common patterns, unique outcomes, gaps in the literature, and directions for future research. From this volume, it is apparent that disease pathogenesis differs between males and females. There are significant gaps in our understanding of the precise mechanisms mediating sex-biased immune responses. Future research must continue to define the pathways mediating how hormones, genes, and the microbiome alter the functioning of cells and tissues to cause profound differences in the pathogenesis of infectious disease and efficacy of treatments for these diseases. The ability to gather and analyze large data sets such as genomes, transcriptomes, proteomes, metabolomes, and microbiomes is important in most areas of biology, including the study of sex differences. We believe that consideration of sex-based differences

will lead to optimization of medical interventions for individuals. Making sure that these data are stored and made publically accessible will be of critical importance. Many of our biological differences are hardwired in our genome, but our biotic and abiotic environment may affect the kinetics, magnitude, and skewing of these differential responses when faced with immunological challenges. We also cannot ignore the profound effects that gender (i.e., the constellation of sociocultural factors) can have on the outcome of infectious diseases and how this interacts with our biology. The concept of personalized medicine is not novel; what is novel is that sex may be a fundamental factor to consider when designing and administering treatments for infectious diseases. We will only achieve this end result if journals and funding agencies continue to require that investigators report the sex of their cells, animals, and subjects and disaggregate and analyze data by sex/gender.

References

Beery AK, Zucker I (2011) Sex bias in neuroscience and biomedical research. Neurosci Biobehav Rev 35(3):565–572

Bolnick DI, Snowberg LK, Hirsch PE, Lauber CL, Org E, Parks B, Lusis AJ, Knight R, Caporaso JG, Svanbäck R (2014) Individual diet has sex-dependent effects on vertebrate gut microbiota. Nat Commun 5:4500. doi:10.1038/ncomms5500

Chaban B, Links MG, Jayaprakash TP, Wagner EC, Bourque DK, Lohn Z, Albert AY, van Schalkwyk J, Reid G, Hemmingsen SM, Hill JE, Money DM (2014) Characterization of the vaginal microbiota of healthy Canadian women through the menstrual cycle. Microbiome 2:23. doi:10.1186/2049-2618-2-23

Clayton JA, Collins FS (2014) Policy: NIH to balance sex in cell and animal studies. Nature 509 (7500):282–283

FDA (2013) Zolpidem containing products: drug safety communication-FDA requires lower recommended doses. FDA

Flórez-Vargas O, Bramhall M, Noyes H, Cruickshank S, Stevens R, Brass A (2014) The quality of methods reporting in parasitology experiments. PLoS ONE 9(7):e101131. doi:10.1371/journal.pone.0101131

Guidelines FG (1977) General considerations for the clinical evaluation of drugs

Klein SL, Roberts CW (eds) (2010) Sex hormones and immunity to infection. Springer, Berlin

Parekh A, Fadiran EO, Uhl K, Throckmorton DC (2011) Adverse effects in women: implications for drug development and regulatory policies. Expert Rev Clin Pharmacol 4(4):453–466. doi:10.1586/ecp.11.29

Pinn VW (1994) The role of the NIH's office of research on women's health. Acad Med 69 (9):698–702

Simon V (2005) Wanted: women in clinical trials. Science 308(5728):1517

Stanberry LR, Spruance SL, Cunningham AL, Bernstein DI, Mindel A, Sacks S, Tyring S, Aoki FY, Slaoui M, Denis M, Vandepapeliere P, Dubin G (2002) Glycoprotein-D-adjuvant vaccine to prevent genital herpes. N Engl J Med 347(21):1652–1661

Yoon DY, Mansukhani NA, Stubbs VC, Helenowski IB, Woodruff TK, Kibbe MR (2014) Sex bias exists in basic science and translational surgical research. Surgery 156(3):508–516. doi:10.1016/j.surg.2014.07.001

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S.L. Klein, C.W. Roberts (eds.), *Sex and Gender Differences in Infection and Treatments for Infectious Diseases*, DOI 10.1007/978-3-319-16438-0