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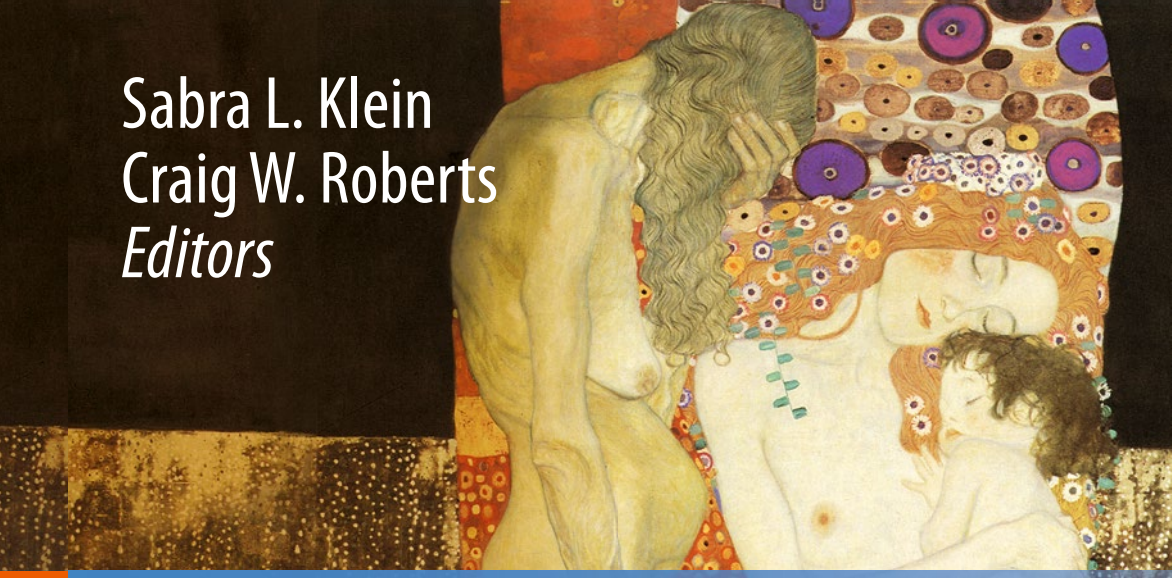
Editors



Sex and Gender Differences in Infection and Treatments for Infectious Diseases

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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.

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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.

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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.,

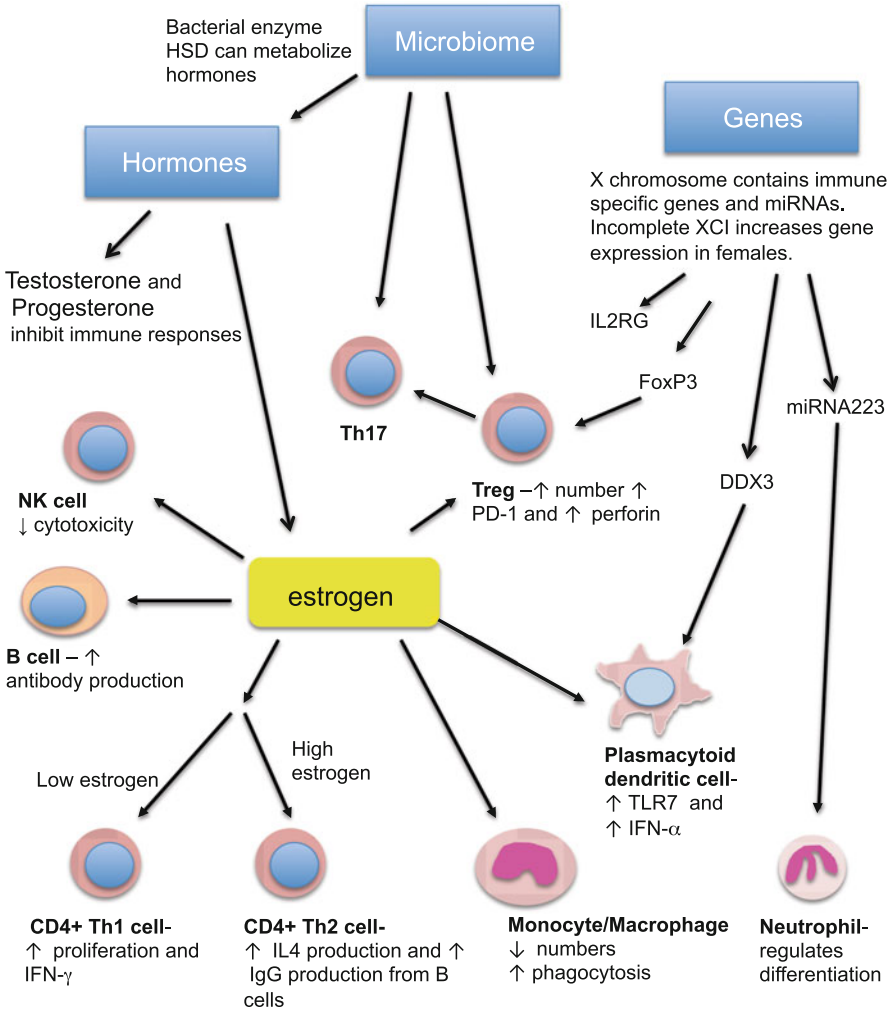

Fig. 1.1 Sex-specific differences influence the immune response. Hormones, genes, and the microbiome all influence the immune response. Estrogens have direct effects on immune cells. Bacterial hydroxysteroid dehydrogenase (HSD), microRNAs (miRNA)

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).