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Sex Drives Dimorphic Immune Responses to Viral Infections

Soumitra Ghosh* and Robyn S. Klein*,†,‡

New attention to sexual dimorphism in normal mammalian physiology and disease has uncovered a previously unappreciated breadth of mechanisms by which females and males differentially exhibit quantitative phenotypes. Thus, in addition to the established modifying effects of hormones, which prenatally and post-pubertally pattern cells and tissues in a sexually dimorphic fashion, sex differences are caused by extragonadal and dosage effects of genes encoded on sex chromosomes. Sex differences in immune responses, especially during autoimmunity, have been studied predominantly within the context of sex hormone effects. More recently, immune response genes have been localized to sex chromosomes themselves or found to be regulated by sex chromosome genes. Thus, understanding how sex impacts immunity requires the elucidation of complex interactions among sex hormones, sex chromosomes, and immune response genes. In this Brief Review, we discuss current knowledge and new insights into these intricate relationships in the context of viral infections. *The Journal of Immunology*, 2017, 198: 1782–1790.

Age, sex, and immune state of the host are considered salient biological factors that determine the extent and strength of pathogen clearance during infectious diseases (1–3). With regard to viral infections, epidemiological studies revealed that males have a higher mortality compared with females, who reportedly display stronger antiviral cellular and humoral immune responses (4). Although stronger immune responses may provide better protection against certain pathogens, in some chronic viral infections it can lead to aberrant antigenic responses with immunopathology (5, 6). Net sex differences or the degree of sexual dimorphism in biological responses derive from genetic differences in chromosome complement and induce their effects via acute activational or prenatal organizational/epigenetic effects of gonadal sex hormones (estrogen, progesterone, and androgens), extragonadal effects of sex chromosome-encoded

genes, and compensatory mechanisms, such as reduction in gene-dosage differences through X-chromosome inactivation. These processes underlie sex differences in most tissue responses during normal and disease states, including immunological responses to infectious diseases (Fig. 1).

Differences in susceptibility and response to viral pathogens observed in males and females have primarily been attributed to activational effects of sex hormones and dosages of genes on the X and Y chromosomes (4). The onset of puberty is associated with the numbers and functions of circulating granulocytes and monocytes, which are decreased, but activated, in females as a result of rising levels of progesterone in the setting of ovulation or pregnancy (7, 8). In addition, myeloid cells and lymphocytes express receptors for estrogen, progesterone, and androgens, which orchestrate transcriptional pathways and ligand-dependent or ligand-independent signaling cascades that influence innate and adaptive immune responses to viruses (9–12). With regard to gene dosage on sex chromosomes, X-linked genes, such as *IL-13*, *IL-4*, *IL-10*, *XIST*, *TLR7*, *FOXP3*, and sex-determining region Y box 9 (*SOX9*), on the X chromosome and sex-determining region Y (*SRY*; testis-determining factor) and *SOX9* on Y chromosome may underlie sexually dimorphic responses that contribute to stronger innate, cellular, and humoral immune responses and susceptibility to autoimmune diseases in females compared with males (13–15). Studies in humans and animal models indicate sexually dimorphic mechanisms that contribute to virologic control during infections with HIV-1, vesicular stomatitis virus, hantavirus (Seoul virus), influenza virus (H1N1), hepatitis C virus, Theiler's murine encephalomyelitis virus, HSV-1 and coxsackievirus B3 (CVB3) (5, 16–20). In this review, we introduce the various mechanisms that impose sexual dimorphism in immune function in males and females. We then discuss the impact of sexually dimorphic immune responses on pathogenesis during viral infections.

Organizational effects of sex hormones on immune function

The initiation of sexual dimorphism occurs through early embryonic development due to effects of genes on sex chromosomes (21, 22). The *SRY* gene on the Y chromosome

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Abbreviations used in this article: AR, androgen receptor; CVB3, coxsackievirus B3; E2, 17 β -estradiol; E3, estriol; ER, estrogen receptor; FCG, four-core genotype; MCMV, murine CMV; pDC, plasmacytoid dendritic cell; RSPO, R-spondin; SLE, systemic lupus erythematosus; *SOX9*, sex-determining region Y box 9; *SRY*, sex-determining region Y; Treg, regulatory T cell.

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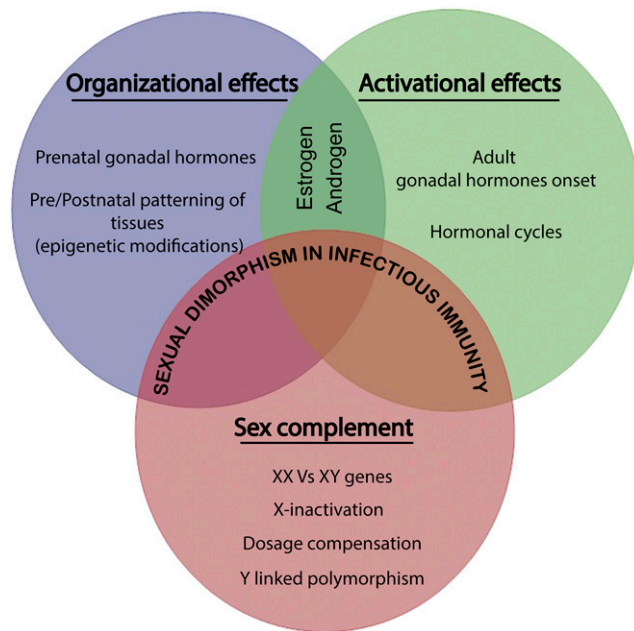


FIGURE 1. Mechanisms of tissue sexual dimorphism that underlie sex differences in immune responses. Sex hormones, such as estrogens and androgens, contribute to organizational and activational effects via gene effects that include promoter activation and chromatin remodeling (epigenetic modifications). Sex chromosome complement exerts its effect in promoting sexual dimorphism independent of sex hormones. X-dosage compensation and escape from X-inactivation influence differential gene expression of innate immune molecules. Y chromosome contributions include Y gene-associated polymorphisms. Studies evaluating sexual dimorphism in immune responses focus on the interdependence of these factors, as well as their independent contributions.

induces male supporting cell precursors to differentiate into testosterone-expressing Sertoli cells, leading to masculinization of all tissues within the developing embryo (23–25). During embryonic development, the increased expression of multiple genes maintains the XX sex phenotype and inhibits the expression of genes, such as SOX9, a transcription factor that favors the XY male phenotype (15). In males, expression of SOX9 protein downstream of *Sry* is crucial in inducing male sex phenotype. SOX9 can independently induce male-to-female sex reversal in male embryos through deletion from chromosome 17 (23, 26, 27). Gonadal R-spondin (RSPO)1 is a secreted protein that is highly expressed in female blastocysts and interacts with Wnt4, which promotes ovarian development and estrogen production via the Wnt4/ β -catenin signaling pathway (28, 29). Loss-of-function mutational studies in RSPO1 and RSPO^{-/-} XX mice confirmed this observation (27–30). FOXL2, a forkhead transcriptional regulator, also contributes to ovarian differentiation and maintenance during embryonic development in females via suppression of testis-specific or male sex-determining genes, such as SRY target gene SOX9 and fibroblast growth factor 9, the latter of which represses Wnt4 (31–34). Although SOX9 deletion is embryonically lethal, homozygous conditional inactivation and mutational studies of SOX9 confirmed that SOX9 induces suppression of Wnt4, Foxl2, and β -catenin to prevent feminization of the male embryo (15). In addition, SOX9 increases the expression of genes required for male phenotype development in mammals, including steroidogenic factor1, doublesex and mab-3 related transcription factor 1, and GATA4-binding protein (23, 31, 35, 36).

Studies in rodents show that sexual dimorphism in immune function occurs through embryonic development and is maintained postnatally via the actions of gonadal hormones, such as estrogen and testosterone (37–39). Prenatal castration of male mice results in postpubertal thymic involution and aberrant T cell subset differentiation (40). Females exposed to higher concentrations of androgen prenatally as a result of congenital adrenal hyperplasia exhibit less modeling of behavior shown to them by other females, suggesting that gender-related behavior change is due to prenatal hormonal exposure (41). Surprisingly, immune response to heterologous MLR (MLR-A) is unaffected by perinatal masculinization in both sexes and is strongest in unmanipulated females (42, 43). Consistent with this, loss of feminization at prenatal stages leads to decreased T cell/B cell ratios compared with normally feminized mice, whose ratios mirror those observed in male animals (44). Several studies also report the role of gonadotropin-releasing hormone, which maintains early levels of gonadal hormones in both sexes, in the prenatal patterning of the immune system. Postnatal gonadotropin-releasing hormone antagonism, which inhibits expression of gonadal hormones, results in lower numbers of circulating CD8⁺ T and B cells in male rodents and primates (45). Similar studies in female rats showed reduced numbers of CD4⁺ T cells and reduced immune responses of thymocytes and splenocytes to T cell–mediated Ag (46, 47). The effects of estrogen and testosterone on B cell development and differentiation also directly influence the production of IgG (48, 49). Prenatal secretion of testosterone limits the ability of males to produce Igs compared with females, who maintain much higher default plasma anti-DNA Ig levels compared with males (2, 50). Additional studies in seagull chicks reveal a reduced T cell and plasma Ig-mediated immunity in prenatal testosterone-treated chicks compared with control chicks (51). Activational effects of hormones at puberty further enhance sex differences in Ab production that are present at birth (see below), leading to persistently higher humoral immune response in females throughout their lifetime.

Activational effects of hormones on immune function

Postpubertal expression of gonadal hormones leads to acute and reversible effects in adulthood that maintain physical and behavioral sex differences (52). Estrogen and progesterone in females and testosterone in males are the prime gonadal hormones secreted during the activational period. The effect of hormonal secretion during this period is not limited to the reproductive system; it extends to multiple tissues, including those of the immune system (53). The androgen testosterone is synthesized in gonadal and adrenal tissues of males and females but is predominantly converted to estrogens via aromatization in the latter (54). Endogenous estrogens produced in female mammals include estrone, 17 β -estradiol (E2), and estriol (E3). E2 is the predominant form in females and is produced by theca and granulosa cells of the ovaries in premenopausal women. The level of hormones secreted in postpubertal female mammals varies in a cyclic fashion to facilitate ovulation and subsequent pregnancy.

Estrogen receptors (ERs) exist in two forms, ER α and ER β , which bind estrone, E2, and E3 ligands to mediate gene expression. B and T lymphocytes, mast cells, macrophages, dendritic cells, and NK cells predominantly express ER α (55). Hematopoietic progenitor cells express ER α and ER β (56). Based

on studies in ER α - and ER β -deficient mice, ER α appears to be the key regulator in the differentiation of hematopoietic progenitor cells (57). Females produce higher levels of estrogen and regulate ER activation on their immune cells, which helps them to exert a stronger humoral and cellular immune response (37, 58).

ERs play vital roles in several signaling pathways, acting as a signal transduction molecule in calcium regulation across cell membranes and inducing activation of G coupled- and other surface receptors, such as receptors that drive expression of insulin growth factor 1 and activation of ERK/MAPK, protein kinase C, PI3K, and cAMP signaling (59, 60). ER α is also required for proper dendritic cell differentiation and CD40-mediated cytokine production (61). Although ERs can impact signaling pathways in ligand-dependent and -independent manners (62), signaling in immune cells is ligand-dependent, whereas signaling through coactivator-associated arginine methyltransferase 1 is ligand independent. E2 receptor [specifically ER α 46 (63)] mediates anti-inflammatory signaling in monocytes and macrophages through suppression of CXCL8 (63, 64). ERs activate STAT signaling pathways during T and B cell proliferation, maintenance, and activation. Under inflammatory conditions, ER activation also induces NO synthase and IFN- γ expression in T cells. ER ligands also mediate phosphorylation, nuclear localization, and transcriptional activation of STAT1, STAT3, and STAT5 in B cells and circulating monocytes (65, 66). E2 additionally regulates STAT activity by increasing expression of cytokine-inhibitor proteins, such as suppressor of cytokine signaling 1 and 5, in T cells and macrophages (67–70). In macrophages, ER signaling mediates inhibitory responses toward proinflammatory genes regulated by NF- κ B, such as IL-6. In contrast, E2 directly suppresses the expression of CCL2 in leukocytes, leading to a reduction in migration (71, 72).

It is well established that ERs play an important role in promoting sexual dimorphism in the neonatal brain through chromatin remodeling, particularly via promoter methylation and acetylation. This process is mediated by ER α activation and dimerization, followed by nuclear translocation, DNA binding through estrogen-response elements, and recruitment of receptor coactivators and corepressors, such as nuclear receptor corepressors, leading to epigenetic modifications and regulation of downstream transcriptional factors (73–75). Current studies on epigenetics also indicate that methylation of the ER α promoter contributes to sex differences in the brain, as well as maintains sexual dimorphism throughout life by creating methyl marks on the DNA of the individual (76). However, direct evidence for ER- or androgen receptor (AR)-mediated epigenetic modifications that contribute to immune cell differentiation and function has not been found.

In addition to endogenous ER ligands, ligands that effect ER signaling are found in environmental sources, including food (e.g., phytoestrogens) and pharmaceuticals (e.g., tamoxifen, toremifene and raloxifene, which are selective ER modulators). Selective ER modulators have been used as therapeutics in multiple sclerosis, ovarian cancer, breast cancer, and Ebola virus infection to suppress the activity of Th1 cells and induce Th2 cytokine expression (77–81). Further studies are required to understand the impact of environmental and exogenous estrogens, either independently or in combination with other factors, on immune function during exposure to pathogens.

Progesterone also mediates stimulatory and suppressive roles in immune responses. Progesterone receptors are primarily expressed by T and NK cells, but recent studies detected them on dendritic and mesenchymal stem cells, where they suppress Th1 cytokine secretion and increase Th2 cytokine secretion (82, 83). Suppression of T cell cytotoxicity, as well as regulatory T cell (Treg) proliferation, is also mediated by progesterone (84). Progesterone also inhibits the activity of NK cells via downregulation of IFN- γ secretion (85). In macrophages, progesterone suppresses NO levels and inhibits Fc γ R expression and microparticle release, thereby dampening the initial immune response at initial stages of infection. During pregnancy, progesterone enhances immunomodulatory functions of mesenchymal stem cells through upregulation of PGE-2 and IL-6. This is essential in females to maintain the fetal–maternal interface (83).

Most testosterone (98%) is irreversibly converted to an active metabolite, dihydrotestosterone (86), which binds with higher affinity than testosterone to ARs, which are expressed at various levels by leukocytes (87). In innate immune cells, such as neutrophils, AR signaling maintains cellular differentiation via induction of G-CSF signaling through activation of ERK1/2 and STAT3 (88). In wound-healing studies, AR regulates the chemotactic ability of macrophages through upregulation of CCL2, TNF- α , and CCR2 (89). AR signaling also regulates T and B cell function and development. CD4⁺ thymocytes express lower levels of inducible AR, whereas CD4⁺ CD8[−] and CD8⁺ thymocytes express the highest levels (90). Although AR signaling promotes Th1-mediated T cell immune response, it also acts as an antagonist to NF- κ B and IFN type I signaling pathways (91). Specifically, splenic CD4⁺ and CD8⁺ T cells express inducible AR that binds to testosterone in males (92). Castration studies revealed that, in the absence of testosterone, AR signaling suppresses the activation of CD4⁺ and CD8⁺ T cells via overproduction of IL-2 and IL-2R (47, 93). Moreover, the absence of testosterone leads to dampening of Th1 differentiation from naive CD4 T cells in autoimmune disease conditions through suppression of IFN- γ and IL-2 expression in males (94, 95).

Sex chromosome complement and immune function

Sex chromosome complement arises from fundamental genetic differences in XX and XY cells that are mediated by several processes, such as X chromosome inactivation, X gene dosage, and epigenetic modifications (96). To maintain X gene dosage in the female blastocyst, one of the X chromosomes is inactivated by formation of Barr bodies in individual cells (97). Barr bodies are formed as a result of Xist gene expression and histone modification, making one of the X chromosomes inactive in every cell (98). This process, which is exclusive to XX somatic cells, is random and leads to cells with active maternal or paternal X and compensates X gene dosages in all somatic cells (14). The gene products of X or Y gene also facilitate epigenetic modifications on the DNA of an individual. SRY interacts with Kruppel-associated box domain transcriptional factor through the high mobility group box on SRY to recruit histone-modifying enzymes, such as histone deacetylase and heterochromatin protein 1, which remodels chromatin (99, 100). Few studies evaluated sexual dimorphism in chromatin remodeling, irrespective of hormonal

influence, and those that did focused on the CNS. Thus, H3 methylation is more prevalent in cortical regions of the brain in males compared with females (101), and histone deacetylase 2 and 4 bound more prevalently to promoters of *Esr1* and *Cyp19a* during brain differentiation in males compared with females. This increased interaction leads to stronger deacetylation and higher gene expression (102).

microRNAs are also sexually dimorphic in nature and regulate SRY-related genes. For example, in the prenatal stage, miR-124 is highly expressed in female-supporting cells and suppresses *SOX9* gene expression. In contrast, miR-202-5p/3p is highly expressed in males through *SOX9*, which is generated by male-supporting cells, and is necessary for male sex determination. Data also suggest that miR-202-5p is a direct transcriptional target of *SOX9* during testis differentiation (103). Interestingly, miR-124 also was shown to inhibit STAT3 signaling, which suppresses T cell proliferation and leads to *Foxp3*⁺ Treg induction, including upregulation of IL-2, IFN- γ , and TNF- α (104, 105). Another study using a murine model of multiple sclerosis found that peripheral administration of miR-124 leads to systemic deactivation of macrophages, reduced activation of myelin-specific T cells, and suppression of disease progression (106). Because research regarding the role of sex chromosome complement in many physiologic and disease processes is still in its early stages, less is known about their contributions to antiviral immunity. However, studies using four-core genotype (FCG) mice, in which the role of XX versus XY genes can be separated from those of gonadal hormonal effects, are providing new insights into the impact of sex chromosome complement on immune responses, particularly during autoimmune diseases.

Female bias in disease expression of autoimmunity is well established, with female/male ratios in systemic lupus erythematosus (SLE) and multiple sclerosis approaching 4:1 and 9:1, respectively. A study of sex chromosome aneuploidy in male subjects expressing an excess X chromosome found that they were at a higher risk for SLE (107). Thus, the X chromosome likely plays a crucial role in disease incidence independently of hormonal effects (107, 108). The FCG mouse model is a novel tool for examining the effect of sex chromosome complement XX and XY on phenotypic sex differences induced in male and female mice without the confounding effects of hormonal patterning. In the FCG model, the testis-determining gene *Sry* is deleted from the Y chromosome on male B6 or SJL mice (XY⁻), and a transgenic *Sry* is inserted at multiple sites on an autosome (109). The model generates four genotypes of mice; the *Sry* transgene is present in XX and XY⁻ mice, which develop testis in contrast to XY⁻ mice, which develop ovaries similar to XX mice (109). Gonadectomy of FCG mice allows further examination of the genetic contributions of sex chromosomes in adult animals that do not express sex steroids.

Using FCG mice, XXSry mice were found to display increased susceptibility for SLE and experimental autoimmune encephalomyelitis compared with XY⁻ Sry mice. Because both XXSry and XY⁻ Sry mice had testes during development, and the only difference between the two groups is the presence of the Y chromosome complement, this study confirmed that sex complement alone could promote susceptibility to diseases, irrespective of the hormonal secretions (52, 108). Further comparison between XX and XY⁻ mice found higher levels of

IL-13R α 2 and reduced levels of Th2 cytokines in spleen cells isolated from XX mice. These results suggest that subdued Th2 cytokine levels that result from an increase in X linked gene IL-13R α 2 expression could be due to X chromosome complement (108).

Sexually dimorphic immunity to viral infections

Differences in susceptibility to viral infections are likely due to inherent differences in the immune system of females and males. Females mount a stronger immune response to viral infections compared with males as the result of more robust humoral and cellular immune responses (Fig. 2). Clinical studies of viral infections in humans are complicated by the impact of nonbiological factors, such as exposure rates, social behavior, habitat and diet, on viral pathogenesis in a sex-specific fashion. However, studies in a controlled setting suggested that levels of estrogen and testosterone differentially alter the expression of genes involved in innate immunity, such as those encoding TLRs and IFNs, in females and males, thereby contributing to sexual dimorphism in viral infections.

Immune response to viral infections

The innate immune response is the first line of defense against any viral infection. Males and females exhibit a different pattern of response to viral infections. The innate response is primarily mediated by three classes of pattern recognition receptors (PRRs): TLRs, retinoic acid-inducible gene I-like receptors, and nucleotide oligomerization domain-like receptors (110, 111). These PRRs detect viral components, such as genomic DNA, dsRNA, ssRNA, RNA with 5'-triphosphate ends, and viral proteins. TLRs and retinoic acid-inducible gene I-like receptors specifically regulate the production of type 1 IFNs and other cytokines. In contrast, nucleotide oligomerization domain-like receptors regulate

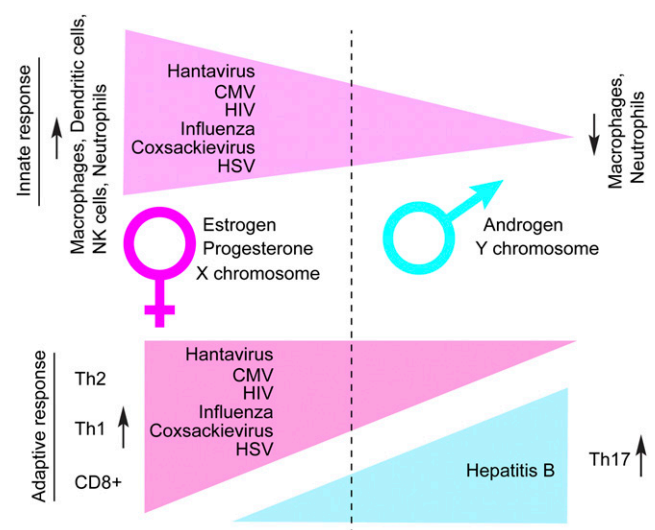


FIGURE 2. The interplay of sex chromosomes, sex hormones, and immune responses influence sex differences in virologic control. Females display increased innate and adaptive immune responses to most viral infections compared with males as a result of differences in the effects of sex hormones (estrogen, progesterone, and androgen). X and Y chromosome complement also contribute to sexually dimorphic immune responses to viruses in females and males. The relative increase in immune responses in females may contribute to differential levels of virologic control during acute infections and/or immunopathologic effects of antiviral T cells that may lead to chronic inflammation

IL-1 β through caspase-1 activation (110, 112, 113). Sexual dimorphism is observed during antiviral responses mediated by TLR and IFN pathways (114, 115). Immune cells in females exhibit a 10-fold higher expression of TLRs compared with males (116). In mammals, the number and activity of innate immune cells, such as monocytes, macrophages, and dendritic cells, are higher in females than in males. As a result, responses to Ags, vaccines, and infections are also higher in females compared with males (3, 117). The adaptive immune response also exhibits many sexual differences in response to viral infections. Depending on the stage of the infection, females exhibit higher inflammatory Th1 and anti-inflammatory Th2 compared with males (3). Additionally, upregulation of anti-inflammatory genes and higher cytotoxic T cell activity are observed in females. Some studies also showed higher numbers of Tregs in females compared with males. Clinical investigations in humans also reported lower instances of CD3⁺, CD4⁺, and CD4⁺: CD8⁺ ratios in Th cells in males compared with females (44, 118).

DNA virus members of the Herpesviridae family

Like many DNA viruses, herpesviruses replicate within host cell nuclei, with their genome persisting as an episome for the life of the host. In this study we will discuss sexually dimorphic responses to herpesvirus infections that have been traced to activational effects of gonadal hormones.

HSV-1 and HSV-2. Infection with HSVs, DNA viruses that cause oral and genital herpes, and rare encephalitis in immunocompetent individuals can lead to devastating disease in neonates and immunocompromised patients via extensive dissemination to visceral organs and the CNS (119). In males, the T cell-suppressive effects of androgens appear to protect against inflammatory-mediated demyelination infected with HSV-1 (120). Studies in mice showed that E2 treatment increases the chances of survival and decreases vaginal pathology and inflammation in HSV-1-infected females (121). Consistent with this, females infected with HSV generate higher levels of HSV-specific IgG and IgM compared with males (122). Similarly, females infected with HSV-2 are protected against neurologic damage and viral reactivation via virus-specific CD8⁺ T cell activation (123, 124). Studies in ovariectomized mouse models indicated that progesterone treatment increases the susceptibility of females to genital HSV-2 infection, whereas estrogen treatment helps to clear the infection rapidly. Interestingly, combined treatment with estrogen and progesterone in ovariectomized mice resulted in increased infection spread that was accompanied by persistent inflammation and neutrophil infiltration (125, 126). Although studies do not directly indicate the organizational effect, the effect of estrogen and progesterone in ovariectomized animals suggests an effect of sex hormones in HSV-2 infection.

CMV and murine CMV. CMV, a member of the Herpesviridae family with a large genome, causes systemic viral infections in immunocompetent individuals that may be devastating and life-threatening in the immunocompromised (127). Knowledge of sexual dimorphism in CMV infection stems from experiments in murine CMV (MCMV), which is genetically similar to CMV and has been used to study the pathogenesis of CMV in mouse models. In MCMV-infected female mice, IFN- α/β production by splenic plasmacytoid dendritic cells (pDCs) controls viral replication and is required

to prevent viral reactivation (128). Studies in MyD88^{-/-} mice infected with MCMV showed suppression of TLR9 signaling in neutrophils of female mice, which was associated with increased viral replication (129). Additionally, CD4⁺ T cell-mediated responses, including expression of TNF- α , IL-12, IL-6, and IFN- γ , which are required for clearance of CMV, are also mediated primarily by TLR9 signaling, which is decreased in females (130, 131).

RNA viruses

RNA viruses may replicate in either the nucleus or the cytoplasm and, except for HIV-1, are generally cleared by host adaptive immune responses. In this article we outline sexually dimorphic immune responses to clinically relevant RNA viruses.

Hantavirus. Hantavirus is a negative-sense RNA virus of the Bunyaviridae family that predominantly infects rodents. Airborne transmission of virus occurs in humans by exposure to rodent urine, feces, and saliva and leads to hantavirus pulmonary syndrome, a severe respiratory disease that may be fatal (132). Male rats infected with the Seoul virus strain exhibit higher viral burdens in target organs and shed virus for a longer duration than do similarly infected female animals (16, 133, 134). Accordingly, antiviral and proinflammatory factors, such as *Tlr7*, *MyD88*, *Ifn- β* , *TNF- α* , and *Ccl5*, are more highly expressed in female rodents (16, 135). Consistent with this, acute infection with Puumala virus strain in humans is associated with higher concentrations of IL-9 and GM-CSF in females compared with males (136, 137).

CVB3. Coxsackieviruses (genus *Enterovirus*) belong to the Picornaviridae family of positive-sense ssRNA viruses. CVB3 infection leads to myocarditis during the acute and chronic phases, which affects more males than females, with double the mortality in infected individuals under the age of 40 (138). Cardiomyocytes are directly infected by CVB3 during the acute phase, which is followed by a chronic phase with a prolonged T cell-mediated immune response and persistence of CVB3 within the heart (139). Coxsackievirus AR is required for CVB3 entry into cardiomyocytes (140). Chronic-phase CVB3 myocarditis is an autoimmune disease that requires Th17 cells and whose differentiation and expression of IL-17 are suppressed by estrogen, making females less susceptible (138, 141). In contrast, lack of estrogen and the presence of testosterone induce Th17 cell differentiation in CVB3-infected males, enhancing autoimmune-mediated cardiac damage. In females, Th2- and Treg-mediated immune responses, which are increased through ER α signaling in T cells and macrophages in heart tissues, suppress CVB3-mediated immunopathology while clearing infection (139, 142). A recent study also indicates that sex chromosome complement plays a significant role in survival from CVB3 infection. Survival of CVB3-infected B6-ChrY consomic male mice was exclusively dependent on the Y^{CVB3} loci on chromosome Y and independent of prenatal or adult testosterone (24). In summary, the immune make-up of the males and the presence of male sex steroid testosterone promote the spread of CVB3, leading to myocarditis. Sex complement, as well as the activational effect of hormones, contributes to CVB3 pathogenesis.

Influenza. The incidences of H5N1 avian influenza and H1N1 and H2N2 pandemic influenza, RNA viruses that cause severe, inflammatory-induced respiratory diseases, all demonstrate

significant sexual dimorphism, affecting more females than males (143, 144). Reports on H1N1 pandemic influenza indicated higher mortality in females of reproductive age (20–49 y), implicating roles for female gonadal hormones, especially during pregnancy (145). In murine studies, H1N1 infection in female mice prolongs the diestrus cycle, leading to lower serum levels of E2 (5, 145, 146). This reduction in E2 significantly increases the expression of inflammatory cytokines and chemokines, including TNF- α , IFN- γ , IL-6, and CCL2, the latter of which promoted the influx of mononuclear cells into virally infected lungs and exacerbated immune responses (5, 147). Under chronic infection, low levels of E2 bind to ER- α and modulate NF- κ B transcriptional activity, further contributing to augmented inflammatory responses and immunopathology (114, 145). In contrast, lower levels of estrogen and higher levels of androgen in influenza-infected males were linked to immunosuppression and reduced T cell counts, which may limit immunopathology in males (148). Clearly, more studies emphasizing the interplay among sex, hormones, and genes are required to further understand the effect of sexual dimorphism on influenza pathogenesis.

HIV-1. Untreated HIV-1 infection leads to AIDS, with near-complete loss of CD4⁺ T cells. Meta-analysis data show 41% less HIV RNA in women compared with men following primary infection, which gradually increase to a higher viral load set point compared with males after chronic infection (149–151). In females, HIV-1 promotes type 1 IFN production by pDCs via TLR7 signaling, which itself is increased downstream of E3 signaling (115, 152). These effects lead to strong, initial cellular responses that limit HIV-1 replication (153). However, continuous production of type 1 IFN by pDCs in females leads to chronic T cell activation with CCR5 expression, providing more targets for HIV-1 (154). In HIV-1-infected women, pDCs produce more IFN- α through TLR7 ligand activation. This results in a secondary activation of CD8⁺ T cells (115). Follow-up studies showed that, even with the same viral load, pDCs obtained from females showed higher CD8⁺ T cell activation compared with men (115). Additionally, females mount a stronger B and T cell activation following HIV-1 infection as a result of the higher baseline count of CD4⁺ T and CD8⁺ T cells compared with men (115, 151). The concentration of estrogen-binding SHBG is increased in HIV-infected males. Although the mechanism is not well understood, suppressing SHBG levels decreases the severity of HIV infection in male patients (155, 156). Another recent study in SCID mice showed that ERs ESR1 and ESR2 and X chromosome complement in females are necessary for IFN- α and TNF- α production under TLR7 activation in pDCs (157). This study confirmed that estrogen, female sex hormones, and X chromosome dosage confer stronger innate and adaptive immune responses in females compared with males in HIV infection. Altogether, these studies suggest that estradiol treatment could facilitate a stronger immune response to HIV infection.

Although sexual dimorphism is observed in HIV pathogenesis, studies are needed to better understand the underlying mechanisms that contribute to sex-based immune cell regulation in HIV-1-infected patients. These will assist in the design of experiments and the accuracy of clinical trials in HIV-1-infected populations.

Vaccine for viral infections

A sexually dimorphic response to viral vaccines is observed in males and females. Viral vaccines against hepatitis A, hepatitis B, and HSV-2 all exhibit a stronger side effect in young females following immunization. Higher IgA and IgG Ab responses, as well as higher transcriptional activation of genes important in immune cell signaling, such as IFN and TLR7, are also observed in females compared with males (143, 158). These studies clearly demonstrate that the development of vaccines against viral infection should carefully consider the effect of differential immune responses in males and females.

Conclusions

This review highlights early and recent findings regarding the impact of sex on immune cell numbers and function, with a focus on sexually dimorphic phenotypes during viral infections. Although still an emerging field, it is clear that mechanisms by which mammals achieve and maintain sex differences can directly and indirectly influence host–pathogen interactions at the cellular and molecular levels. These findings support the notion that there are, in fact, “two normals” with regard to manifestations of infectious diseases, with sex-specific responses during acute virologic control and in immunopathologic manifestations of viral infections. Accordingly, the development of therapeutics to treat various phases of viral infections will require continued study and comparisons of viral pathogenesis in female and male animals and humans.

Disclosures

The authors have no financial conflicts of interest.

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