Sex-Based Differences in HIV Type 1 Pathogenesis

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Human immunodeficiency virus (HIV) remains a global infectious diseases threat that disproportionally affects women. Beyond social and political factors, biological and genetic differences have been identified that lead to differential disease courses and outcomes in men and women. Following HIV type 1 (HIV-1) seroconversion, women have up to 40% lower HIV loads and higher CD4+ T-cell counts than men. However, at the same level of viremia, progression to AIDS is faster in women. After adjustment for viral load, HIV-positive women also display increased levels of generalized immune activation and experience the consequences of elevated inflammatory activity more frequently than men. Part of these observations are linked to sex-based differences in innate immunity, in which the differential ability of plasmacytoid dendritic cells to produce interferon α following stimulation of Toll-like receptor 7 and upregulation of interferon-stimulated genes play a central role. Here, we review the current knowledge and remaining gaps therein regarding sex-based differences in HIV-1 pathogenesis.

Keywords. HIV-1; sex; Immune activation; interferon-alpha; ISG.

Differential susceptibility to and outcomes of infections between women and men have been well documented in the literature for a variety of pathogens [1]. Sexually transmitted infections caused by viruses, including herpes simplex virus type 2, human papillomavirus, and human immunodeficiency virus type 1 (HIV-1) are among the most prevalent infections worldwide and are known to disproportionately affect women. Despite major advances in treatment and prevention of HIV-1 infection over the past 3 decades, the AIDS pandemic and the >35 million people living with HIV-1 remain among the major health challenges of our time [2]. In sub-Saharan Africa, the epicenter of the global epidemic, women still account for approximately 60% of all people living with HIV-1, and young women aged 15–24 years represent a particularly vulnerable population for HIV-1 acquisition, accounting for more than a quarter of new infections. Gender inequalities and harmful gender norms that promote unsafe sex and limit access to health services for HIV-1 infection and reproduction continue to drive the HIV-1 epidemic in many countries.

Gender inequalities include vulnerability to rape, sex with older men, and unequal access to education and economic opportunities. In comparison to men, women are more likely to acquire HIV-1 at an early age, resulting in a global HIV-1 prevalence among girls and young women that is double or greater than that among males of the same age [2]. While multiple social, political, economic, and legal issues require attention and urgently need to be addressed to ensure decreased HIV-1 acquisition rates among females at risk, increased access to care, and improved health outcomes for women living with HIV-1 [2], we will herein focus on reviewing the biological factors influencing the differential outcomes of HIV-1 infection in women and men, with particular focus on genetic and immunological factors.
of steroid hormones, to differences in anatomy, and to X and Y chromosome-linked factors, such as X chromosome inactivation or genes encoded on the Y chromosome. It has been well established through cross-sectional and longitudinal studies in HIV-1 infection that in acute [3–7] and chronic HIV-1 infection [8, 9], women have lower plasma viral loads and higher CD4+ T-cell counts than men. It has been shown that HIV-1 loads in untreated women are up to 40% lower than those in males; however, despite harboring lower viral loads for a given level of viremia, women had a 1.6-fold higher risk of progressing to AIDS [10], ultimately resulting in similar times to AIDS between infected women and men. Despite these sex-specific differences observed in HIV loads, their impact on the establishment and maintenance of HIV latency and the nature of the viral reservoir remains unknown, warranting further investigation (Table 1).

It has been suggested that women experience or report fewer symptoms during primary infection, which may delay diagnosis in this population [7]. Meditz et al also demonstrated in a large cohort of North American HIV-1 seroconverters that women experienced >2-fold more combined HIV- and AIDS-related illnesses than men and that the morbidity was particularly increased among nonwhite women. However, in this study the authors suggest that part of the observed sex-based differences in morbidity were not biologically based but resulted from socioeconomic factors and possibly race [7]. Dissecting the reason for the differences in markers of disease progression and the differential risk for progression to AIDS between individuals of opposite sexes has been an intense area of research in recent years. Differences in immunological responses to HIV-1, possibly mediated through the effects of gonadal steroids or as a result of genetic variation, have been implicated as probable mechanisms and are discussed below.

Female Sex Hormones in HIV-1 Infection

Studies in animal models have examined the direct role of sex hormones on acquisition of sexually transmitted infections. While rhesus macaques were found to be more susceptible to intravaginal simian immunodeficiency virus (SIV) infection during the luteal phase (when the progesterone level is high), compared with the follicular phase (when the estrogen level is high), of their reproductive cycle [11] and had enhanced SIV acquisition following administration of depot-medroxyprogesterone acetate (DMPA) [12], the systemic administration of estrogens protected female rhesus macaques against intravaginal challenge with highly pathogenic SIV [13]. In humans, sex hormones have been implicated in modulating HIV-1 acquisition, both during the menstrual cycle and in pregnancy. In a prospective cohort study of discordant couples, pregnancy was associated with increased risks of acquisition among females and of transmission from females to males [14], but recent data from population-based studies did not find these associations and in fact suggested a possibly decreased risk of HIV-1 acquisition during pregnancy [15]. The suggested impact of hormonal contraception on HIV-1 acquisition represents an important public health issue and remains a matter of ongoing controversy. A detailed recent meta-analysis concluded that all reviewed trials studying norethisterone enanthate and most of the studies on oral contraceptives did not demonstrate an increased acquisition risk [16]. The authors suggested that more evidence was needed to draw definitive conclusions on the risk of HIV-1 acquisition associated with the use of injectable DMPA, an inexpensive, effective, female-controlled method of contraception used by millions of women worldwide. This conclusion was based on the heterogeneity of investigative methods and some studies reporting an increased risk of HIV acquisition, with other studies demonstrating no association [16].

Following HIV-1 infection, women with self-reported normal menses were found to have normal levels of progesterone and estradiol during the menstrual cycle, similar to HIV-seronegative controls. HIV-1 RNA levels were shown to vary with the menstrual cycle in the female genital tract but not in the peripheral blood compartment [17] and were more elevated in endocervical canal fluid than in blood plasma [18]. It has also recently been suggested that the female genital tract may serve as a reservoir of persistent HIV-1 replication during highly active antiretroviral therapy (HAART) [19]. Meditz et al recently described increased levels of CCR5 expression on endocervical CD4+ T cells in postmenopausal women, which may increase the risk for HIV-1 acquisition in older women [20]. However, overall the exact interplay between sex hormones, mucosal immunity in the female reproductive tract, and HIV acquisition remains inadequately understood and warrants further investigation to optimally design preventive and therapeutic interventions.

INNATE IMMUNITY AND IMMUNE ACTIVATION IN HIV-1 INFECTION

Sex Differences in the Toll-like Receptor (TLR) Response to HIV-1

It has now been well established that generalized immune activation represents a strong predictor for HIV-1 disease

Table 1. Current Gaps in Knowledge About Sex-Based Differences in Human Immunodeficiency Virus Type 1 (HIV-1) Pathogenesis and Warranted Future Studies

| Identification of molecular mechanisms and pathways leading to sex-based differences in HIV pathogenesis | Analysis of sex-based differences in the establishment and maintenance of HIV latency | Evaluation of the size and nature of HIV reservoirs in women and men | Investigation of sex-based differences in aging and its impact on HIV-1 infection | Impact of menopause on HIV-1 pathogenesis | Analysis of sex-specific outcomes in T-cell regulation | Evaluation of sex-specific outcomes in immunity to HIV vaccines | Understanding the interplay between sex hormones and the microbiome in women and men |

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progression, independent of viral load. Plasmacytoid dendritic cells (pDCs) can contribute importantly to HIV-1–associated immune activation through their ability to sense HIV-1 single-stranded RNA via TLR7 [21, 22]. Building on data by Berghoefer et al, who reported higher production of interferon α (IFN-α) by pDCs from women following stimulation with TLR7 ligands [23], Meier et al demonstrated substantial sex-based differences in the response of pDCs to HIV-1 and showed that pDCs derived from women produced significantly higher amounts of IFN-α in response to HIV-1–derived TLR7/8 ligands or whole inactivated HIV-1 (AT-2 virus), compared with men. These sex-based differences in TLR7-mediated IFN-α production by pDCs furthermore resulted in stronger activation of CD8+ T cells (pDCs) can contribute importantly to HIV-1 progression, independent of viral load. Plasmacytoid dendritic cells (pDCs) can contribute importantly to HIV-1–associated immune activation through their ability to sense HIV-1 single-stranded RNA via TLR7 [21, 22]. Building on data by Berghoefer et al, who reported higher production of interferon α (IFN-α) by pDCs from women following stimulation with TLR7 ligands [23], Meier et al demonstrated substantial sex-based differences in the response of pDCs to HIV-1 and showed that pDCs derived from women produced significantly higher amounts of IFN-α in response to HIV-1–derived TLR7/8 ligands or whole inactivated HIV-1 (AT-2 virus), compared with men. These sex-based differences in TLR7-mediated IFN-α production by pDCs furthermore resulted in stronger activation of CD8+ T cells in vitro. The percentage of IFN-α–expressing pDCs following stimulation with HIV-1–derived TLR7/8 ligands was significantly correlated to plasma progesterone levels in study participants, suggesting that sex hormone levels can modulate the ability of pDCs to produce IFN-α [24]. In line with this observation in humans, recent studies by Seillet et al that used humanized mice demonstrated that 17β-estradiol can indeed enhance the ability of pDCs to respond to TLR7 stimulation [25]. The sex-based differences in HIV triggered in vitro IFN-α production by pDCs observed in humans were reflected in vivo by higher levels of HIV-1–associated T-cell activation in women, compared with men, after adjustment for viral load. Women had an average of 5% more activated HLA-DR+CD38+CD8+ T cells for a given viral load and reached the same levels of CD8+ T-cell activation as men at approximately 1 log10 lower HIV-1 load. Taken together, these data suggested a critical role of the TLR7/IFN-α pathway in inducing HIV-1–associated immune activation and thereby possibly in the described sex-based differences in the manifestations of HIV-1 disease.

Higher Expression of IFN-Stimulated Genes (ISGs) in HIV-1–Infected Women

To investigate the sex-dependent differences in HIV-1–induced IFN-α production, Chang et al performed expression analysis of 98 genes involved in the TLR and type I IFN signaling pathways on populations of flow-sorted T cells and DCs derived from men and women with untreated chronic HIV-1 infection [26]. In this study, expression of a number of ISGs, including those encoding ISG15, MX1, IFIT3 and IFIT1, IRF7, and OAS2, correlated with markers of disease progression, and after adjustment for HIV-1 load, higher expression of several ISGs was observed in females. These data suggest that HIV-1 infection leads to stronger induction of ISGs in women, compared with men, for the same amount of virus replication and are in line with a number of recent studies suggesting sex-based differences in the IFN-α pathway. This has been specifically illustrated in the context of autoimmunity, such as systemic lupus erythematosus, an autoimmune disease with a female to male predominance of 7:1 and for which it was recently reported that the TLR7 and IFN-α pathways play a critical role [27]. Similarly, hepatitis C virus infection, in which the IFN-α pathways exhibit a central role and IFN-α is part of the currently used treatment regimens, demonstrates a strong sex-based difference in outcome and treatment response, as reviewed by Baden and Rockstroh elsewhere in this supplement.

In recent studies using the LCMV model, IFN-α has been shown to have a differential impact on the outcome of viral infections, depending on whether this pathway is active in the acute or chronic phase of infection [28]. Wilson et al and Teijaro et al demonstrated that blocking of IFN-α during acute lymphocytic choriomeningitis virus (LCMV) infection has detrimental consequences for disease outcome, reflecting the important antiviral activity of IFN-α and IFN-α–induced genes during acute infection [29, 30]. In contrast, during persistent chronic LCMV infection, continuous production of IFN-α contributed to immune pathology, including impairment of T-cell function, and blocking of IFN-α allowed for enhanced immune control and viral clearance. IFN-α may have a similar dichotomous effect during HIV-1 infection, and sex-based differences in IFN-α and ISG induction might explain some of the differences in the clinical manifestations of HIV-1 infection between women and men, including the better control of HIV-1 viremia by women during primary infection (as a consequence of the anti–HIV-1 effect of IFN-α) and the higher level of immune activation and accelerated disease progression in women during chronic infection, after adjustment for the level of viral replication (as a consequence of the proinflammatory effect of persistent IFN-α production; Figure 1).

Clinical Effects of Immune Activation and Premature Aging

Increasing evidence links immune activation in HIV-1 infection to non–AIDS-related comorbidities, cardiovascular events, and premature aging. In the context of the sex-based differences in immune activation described above, it is not surprising that sex plays a role in the sequelae of increased inflammatory activity with differential clinical outcomes. Data from large registry studies recently indicated that relative increases in myocardial infarction rates are higher among HIV-1–infected women relative to noninfected women, compared with HIV-1–infected men relative to noninfected men (relative risk, 2.98 vs 1.40, respectively) [31, 32]. Fitch et al demonstrated that HIV-1–infected women had significantly higher percentages of coronary segments with noncalcified plaque than HIV-1–negative women but also higher percentages than HIV-1–infected and uninfected men [33]. Similarly, immune activation parameters such as soluble CD163 (sCD163), soluble CD14 (sCD14), and the percentage of CD4+HLA-DR+ T cells were significantly higher in HIV-1–infected women than in infected men. In addition, the authors revealed a 2-way interaction between age and sex, demonstrating that sCD163 increased more with age among women than among men [33]. These results are in line with recent data from Martin et al, who investigated markers of innate immune activation in HIV-1–infected women, compared with uninfected
women [34]. Their findings indicated that the plasma levels of the innate immune activation markers CXCL10, sCD163, sCD14, and neopterin and an increased proportion of CD16+ monocytes were elevated in HIV-1–infected women, as opposed to HIV-1–negative controls. The authors concluded from their data that HIV-1 infection confers the equivalent of a 10–14-year increase in the levels of innate immune aging markers, which in turn may contribute to the elevated risk of inflammation-related diseases in HIV-1–positive women [34].

**X CHROMOSOMAL FACTORS ASSOCIATED WITH DIFFERENTIAL HIV-1 DISEASE OUTCOME**

While direct effects of sex hormones may account for many of the described differences in immune function between women and men, some sex-based differences may also be caused by an inherent imbalance in the expression of genes encoded by the X and Y chromosomes. Multiple genes on the X chromosome, 

**Figure 1.** Differential interferon α (IFN-α; green circles) response and IFN-stimulated gene (ISG) upregulation following plasmacytoid dendritic cell (pDC) stimulation with human immunodeficiency virus (HIV) through Toll-like receptor 7 (TLR7) triggering. Beneficial antiviral effects of IFN-α may lead to lower HIV loads and higher CD4+ T-cell counts in females (F) during primary HIV-1 infection. However, in chronic infection, immunomodulatory effects of IFN-α may contribute to the enhanced immune activation and lead to increased sequelae of a chronic inflammatory state in females, compared with males (M).
such as those encoding TLR7 and TLR8, CD132 (IL-2Rγ), FOXP3, and CD40L, regulate immune function and might play an important role in modulating sex-based differences in immune-related diseases [35]. Through a recent microsatellite genome-wide association study of SIV-infected macaques, Sidiqui et al identified an X chromosomal locus as being associated with the rate at which SIV infection leads to the onset of AIDS-related disease in rhesus macaques [36]. On the basis of this finding, the X chromosomal single-nucleotide polymorphism rs968255, located at human Xq.21.1, was identified as a significant genetic determinant of HIV disease progression in females but not in males. Heterozygous female carriers of the C allele demonstrated significantly lower decreases in CD4+ T-cell counts and lower HIV-1 set points than TT homozygous female carriers and males. These data suggested that some differences in sex-based disease outcome in HIV-1 infection may be the result of sex-linked genetic variation and that elucidating the mechanisms and pathways underlying these sex-specific effects may assist in the rational design of biomedical interventions with high efficacy in both sexes [36].

FOXP3, which is encoded by another gene on the X chromosome, is best known for its role as a transcription factor in regulatory T cells, a CD4+ lymphocyte subset involved in the maintenance of peripheral tolerance. More recently, FOXP3 has also been detected in epithelial cells of breast and ovaries and may play a role in carcinogenesis in these organs in females. Some FOXP3 (also known as Scurfin) gene polymorphisms have been associated with autoimmune disease, especially in women [37], and modulation of regulatory T cells (Tregs) through synergistic action of 17b-estradiol and interleukin 6 is thought to be responsible for the sex bias in immune-mediated drug-induced liver injury [38]. In the context of HIV-1 infection, FOXP3 was shown to enhance HIV-1 gene expression by modulating NFκB-p65 binding to the HIV-1 long terminal repeat [39] and may contribute to the promotion of Tregs as part of the HIV-1 latent reservoir [40]. FOXP3-expressing regulatory T cells expand during the late follicular phase of the menstrual cycle, tightly linked to serum levels of estradiol and followed by a sharp decrease in Treg numbers at the luteal phase [41]. Tregs also contribute to establishment of fetal-maternal tolerance during pregnancy. In HIV-1–infected pregnant women immunoregulatory profiles differ from HIV-1–uninfected women [42, 43], an immune unbalance that is thought to interfere with the prevention of fetal rejection and may in part explain the increased risk of abortion in HIV-1–infected women [42].

We and others have recently shown increased frequencies (ie, increased percentages of Tregs among CD4+ cells) in HIV-1–infected individuals [43–45], which may in part be explained by increased thymic output of naïve Tregs [43], peripheral conversion from conventional T cells, or preferential survival of Tregs during HIV-1 infection. This increase in frequencies coincides with a decrease in absolute CD4 Treg counts during chronic HIV-1 infection and is associated with elevated T-cell activation. While some studies suggest decreased levels of Tregs in women, compared with men [46], detailed comparative studies investigating the effect of sex in the context of T-cell regulation of HIV-1 are not available to date. In addition, given the significant fluctuation of Treg levels in fertile women, the menstrual cycle should be taken into consideration when Treg numbers are investigated clinically. Further studies are therefore needed to understand the impact of sex and sex hormones on T-cell regulation in HIV-1 infection and other viral infections, with careful consideration of sex, phase of menstrual cycle, tissue compartment, and age.

**SEX, VACCINES, AND ANTIRETROVIRAL THERAPY (ART)**

Biological differences between the sexes are associated with variations in the immune response to vaccination, and for many vaccines women elicit much stronger humoral immune responses than men, as reviewed by Klein and Pekoz in this supplement issue. However, among the 6 completed phase 2b and 3 HIV vaccine trials [47], study populations in 3 were exclusively or >94% male. The other 3 trials of candidate vaccines recruited both women and men but largely lacked specific analysis for sex-based differences in immunogenicity, adverse events, or protection in the published data. While only the RV144 trial showed marginal overall vaccine efficacy [48], it has been illustrated in other vaccine models that failure to perform analysis with stratification by sex can lead to underestimation of vaccine protection, skewing of immunogenicity data, and an incomplete side effect profile [49].

In HIV-1 drug therapy, it has now been demonstrated that, with equal access to care and correct administration of and compliance with ART, there is no significant sex-based difference in treatment response [50]. Following seroconversion, similar percentages of women and men achieve undetectable viral loads after 6 months of ART [7]. However, multiple studies suggest that side effects of ART and adverse events can differ dramatically between women and men. This was most notable for first and second generation antiretrovirals, when women experienced more frequent occurrences of skin rashes, mitochondrial toxicity, lactic acidosis, gastrointestinal intolerance, and lipodystrophy [51], which led to increased rates of treatment nonadherence among women [52]. However, even clinical studies with newer antiretroviral drugs such as rilpivirine (RPV) and efavirenz (EFV) report sex-based differences in adverse effects. In recent randomized, controlled trials, the incidence of nausea was greater among women than men with regimens containing either EFV or RPV. In contrast, the incidence of diarrhea was greater among men in the EFV group, compared with women, and the incidence of abnormal dreams or nightmares was more frequent among men in both the EFV and RPV groups [53]. A recently published randomized, controlled trial investigated treatment responses to atazanavir plus ritonavir (ATV/r) or EFV in initial antiretroviral regimens.
among females and males, to determine whether treatment outcomes differed by sex. In this study, Smith et al demonstrated that women assigned to receive ATV/r displayed a higher risk of virologic failure than men assigned to receive the same regimen. Self-reported adherence did not differ significantly by sex, but interestingly, females had slower ATV clearance and higher predose levels of ATV, compared with men [54]. Addressing the underrepresentation of women in randomized, controlled trials and dissecting the detailed mechanisms of drug metabolism and toxicities may lead to more and much needed individually tailored and sex-specific approaches to ART and represents an important area for future individualized therapy and personalized medicine.

CONCLUSION

Beyond gender and cultural norms, there are distinct biological differences in the natural history of and immunity to HIV-1 infection between women and men. The persistent higher expression of IFN-α and ISGs in females may in part explain the higher immune activation with subsequent faster disease progression and premature aging observed in chronically infected women, compared with men, for the same level of viral replication. However, X-linked and other genetic associations related to sex-based differences in HIV-1 infection remain incompletely understood and warrant further investigation. A few key gaps in knowledge, including questions relevant to ongoing HIV cure research efforts, aging, and the microbiome, that are in need of further study are summarized in Table 1. It is conceivable that reversal of latency and HIV eradication efforts may well need distinct approaches in women, compared with men. Careful experimental design and dedicated sex-based analysis of HIV clinical and vaccine trial data will be required to gain further insights into sex-based differences in the immune pathogenesis of HIV-1 infection, which will serve as the basis for strategic design of individualized treatment and prevention efforts.

Notes

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