Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Time to get ill: the intersection of viral infections, sex, and the X chromosome

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Females have more robust immune responses than males, and viral infections are more severe for males. Hormones and genetic sex, namely the X chromosome, influence sex differences with immune responses. Here, we review recent findings underlying sexual dimorphism of disease susceptibility for two prevalent viral infections, influenza and SARS-CoV-2, which exhibit male-biased disease severity. Viral infections are proposed to be an initiating event for autoimmunity, which exhibits a female bias. We also review recent work elucidating the epigenetic and genetic contribution of X-Chromosome Inactivation maintenance, and X-linked gene expression, for the autoimmune disorder Systemic Lupus Erythematosus, and highlight the complex considerations required for identifying underlying hormonal and genetic contributions responsible for sex differences in immune responses.

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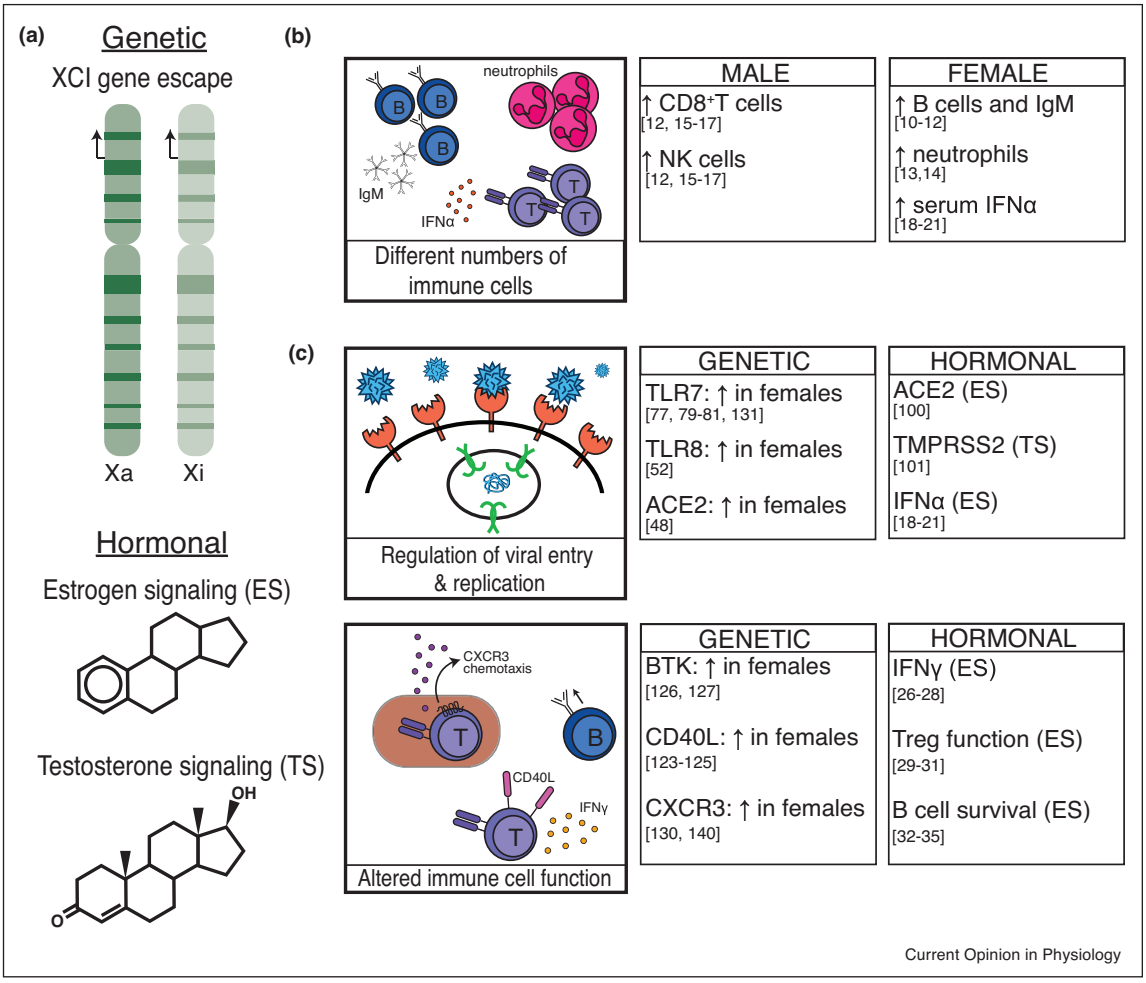
Introduction

Women have more robust immune responses following infection from a variety of pathogens, leading to decreased mortality. Yet this propensity for stronger immune responses may contribute towards increased incidence of autoimmune disease in women. Sexual dimorphism with immune responses originates from genetic and hormonal differences with the immune system, which influence how immune responses to pathogens. Innate immune cells, including neutrophils, natural killer (NK) cells, and macrophages, respond to pathogens by lysing infected cells, phagocytosing infectious particles, and releasing soluble signals such as inflammatory cytokines [1–4]. B and T cells, the major players of the adaptive immune system, become activated in a targeted manner against invading pathogens. B cells produce and secrete specific antibodies that neutralize viral particles and facilitate clearance of the pathogen [1,5]. Upon antigen encounter, some virus-specific B cells enter germinal centers of secondary lymphoid organs to somatically mutate the antigen binding domain and class-switch the constant domains of their immunoglobulin genes [1,5]. This increases the affinity of viral-specific antibodies for the viral antigen and alters effector capabilities of the antibody [1,5]. T cells, either $CD8^+$ or $CD4^+$, can directly lyse infected cells and secrete pro-inflammatory cytokines [1,6]. Two specialized subsets of $CD4^+$ T cells are T follicular helper cells (Tfh), which facilitate B cell affinity maturation, and T regulatory cells (Tregs) suppress aberrant immune responses in the periphery [1,7,8]. The adaptive immune system generates long-lived memory cells that can respond quickly and robustly in the event of a secondary challenge by the same pathogen [1,6,9].

There is sexual dimorphism with immune responses in healthy individuals, with observed differences in numbers of immune cell populations and serum cytokine concentrations (Figure 1). Women have higher numbers of B cells and elevated serum levels of non-class switched antibodies [10–12]. Woman and female rats also have elevated neutrophil counts [13,14] as well as a higher proportion of $CD4^+$ T cells [12,15–17]. In contrast, men have more $CD8^+$ T cells and NK cells [12,15–17]. There are also sex differences with serum cytokine production, in particular Type I Interferons (IFNα) as well as IL-10 [18–22]. These baseline differences contribute to sex biases when the immune system is activated in the presence of a pathogen.

Genetics and hormones contribute to sex differences with immune responses. Sex hormones such as estrogen and testosterone are produced at different ratios, and receptor signaling events regulate a number of important immunity-related genes. Sex hormone signaling can also alter the susceptibility of non-immune cells to viral infection, as well as drive both innate and adaptive immune cell activation and function [23–35] (Figure 1). Estrogen receptor signaling is associated with elevated production of innate pro-inflammatory cytokines, particularly antiviral IFNs [19,21,23]. Elevated estrogen levels during pregnancy and estrus cycles dampen pro-inflammatory responses, demonstrating the complex interactions sex hormones have with immune responses [23]. The genetic basis for sex differences resides with the sex

Figure 1



Genetic and hormonal contributions to sexually biased immune responses. **(a)** Possible factors leading to sexual dimorphic gene expression. **(b)** Differential immune cell populations and soluble mediators in males compared to females. Legend: B cells (dark blue), neutrophils (pink), T cells (purple), IFNα (pink dots). **(c)** Examples of alterations in gene expression driven by genetic or hormonal factors leading to sex-biased immune responses. Legend: Virus (blue), viral receptor (salmon), endosomal TLR7 and TLR8 (green), viral RNA (blue strands), B cells (dark blue), T cells (purple), CD40L (pink, on T cell), CXCR3 (purple dots), IFNγ (orange dots).

chromosomes, and the X chromosome is enriched for important regulatory immune-related genes [36,37]. Female mammals have two X chromosomes and use X-chromosome inactivation (XCI), a hallmark example of epigenetic gene regulation, to equalize X-linked gene expression between the sexes. XCI is established early in female embryonic development and maintained through each cell division through adulthood by various epigenetic modifications, including Xist RNA, repressive histone modifications (H3K27me3, H2AK119-ubiquitin, H4K20me1), the histone variant macroH2a, and DNA methylation, which are enriched across the inactive X (Xi) [38–43]. The Xi is often found near the nucleolus and at the nuclear periphery, which is enriched for heterochromatin [44]. Some genes escape XCI and are expressed from both X chromosomes, leading to altered expression levels between the sexes [45–52]. Here, we will review recent advances investigating sex differences with viral responses to influenza and coronaviruses, and genetic as well as epigenetic contributions to female-biased autoimmunity.

Sex differences with viral infections and vaccinations: influenza and coronaviruses

Sex differences with viral infections and clinical outcomes have been observed for a variety of viruses. Male children under age four had significantly more occurrences of measles, viral meningitis, and hepatitis infections [53]. Male sex in adults was also a significant predictor of severe disease outcome with several viruses, including from hepatitis A, hepatitis B, Epstein-Barr virus, and West Nile virus [54–58]. Men also had higher

levels of HIV RNA in their blood, although when accounting for viral load, women were more likely to develop AIDS [59]. Some viruses display a bias towards more severe disease in female mammals, which might be indicative of heightened antiviral immune responses also causing aberrant immunopathology [58]. Successful viral vaccination strategies rely on the adaptive immune system generating a memory response to inactivated viral particles or viral subunits, and sex biases have also been reported. Women mounted stronger class-switched antibody profiles for vaccinations against yellow fever, measles mumps and rubella, hepatitis A, hepatitis B, herpes, smallpox, and influenza [60–65]. Women also displayed increased local inflammation around the site of vaccination, which may reflect sexual dimorphism with innate immune activation [60,62,65].

Immune responses to viruses exhibit sexual dimorphism, including in viral cell entry, recognition of viral motifs, and immune cell activation (Figure 1). In this section, we highlight recent advances in understanding the underlying sex differences in response to two viral infections that impose great disease burdens worldwide: influenza and SARS-CoV-2. The current mechanistic explanations for sex-biased responses to these infections involve both hormonal and genetic factors as well as interactions between the immune system and other biological processes, serving to highlight the complexities inherent in identifying the underlying causes of sex-biased disease parameters.

Sex differences with influenza infections and vaccines

Seasonal influenza infections result in 3–5 million severe cases and 290 000–650 000 deaths per year worldwide [66] and while many reports do not disaggregate by sex, there is a clear sex bias in influenza susceptibility that changes with age. Young men before puberty exhibit more severe disease compared to age-matched women, which suggests genetic origins for observed sex differences [67,68]. In addition, older men are more likely to be hospitalized versus older women [69]. In contrast, adult pre-menopausal women have increased likelihood of severe illness [67] and increased lung pathology. The picture of sex bias during influenza infection becomes more complicated when examining responses to pandemic influenza outbreaks [70]. For example, in the 2009 H1N1 pandemic, pre-menopausal women had higher rates of hospitalization and also higher risk of death [71–74] ; in contrast, during the 1917–1918 epidemic there was increased mortality for men [75]. Both viral-mediated and immunopathologic lung damage can occur during influenza infection, resulting in the development of Acute Respiratory Distress Syndrome (ARDS) and hypoxemic respiratory failure [76]. Therefore, the increased susceptibility to influenza for women (post-puberty and pre-menopausal) and male-predominance of disease severity particularly with increased age could arise from genetic differences between the sexes in addition to hormonal changes during aging.

Influenza infections in mice also exhibit sex differences. Female mice challenged with the H1N1 strain of influenza produced higher levels of neutralizing and class-switched antibodies compared to male mice [77••]. Higher antibody titers were also observed in female mice following vaccination with inactivated virus, which correlated with higher numbers of germinal center B cells, $CD8^+$ and $CD4^+$ T cells in lymph nodes [77••]. Female immunized mice also had higher transcription of Toll-like receptor 7 (*Tlr7*) and reduced DNA methylation at the *Tlr7* promoter in B cells [77••]. *Tlr7* is X-linked and plays a role in inducing class-switch recombination in B cells [78], thus female-specific elevations with *Tlr7* could explain the higher levels of class-switched antibodies that provide protection from influenza infections (Figure 1**)**. We and others have reported *Tlr7* escape from XCI in human and mouse B cells [79–81], yet additional work is required to establish whether biallelic expression of *Tlr7* provides increased protection from influenza.

Sex hormones also contribute to observed sex differences with immune responses to influenza. Estrogen treatment reduced influenza A replication in human nasal epithelial cells derived from female, but not male, donors, suggesting that estrogen receptor signaling directly affects influenza virus replication [82]. Testosterone also impacts immune responses. Using a machine learning approach, a cluster of lipid metabolism genes regulated by testosterone were identified which correlated with male-specific poor vaccine-induced antibody production [65]. Men with elevated testosterone levels also had the lowest level of antibody production [65]. Yet studies in mice found that testosterone reduced lung inflammation and improved survival following influenza infection, and androgen replacement treatment did not impact the susceptibility of aged mice [83–86]. Additional work is necessary to elucidate the molecular details and species-specificity of sex hormones affecting viral-induced injury and lung repair following influenza infection.

Sex differences with coronavirus and resulting respiratory disease

The current COVID-19 global pandemic is caused by a coronavirus (SARS-CoV-2) and men are more susceptible to infection, severe disease, and mortality [87–89]. Previous outbreaks of the coronaviruses SARS-CoV and MERS-CoV, which resulted in SARS and MERS, also exhibited a male bias for severe disease [90–92]. While epidemiological data from the COVID-19 pandemic is complicated by uneven reporting of sex-disaggregated data and country-by-country testing criterion, a recent analysis from 38 countries identified a male bias for COVID-19 fatalities in 37 countries [89]. For the 12 countries for which the data was available, further breakdown

by both sex and age revealed a significant male bias in case fatality for every age above 30 years [89]. Peripheral blood mononuclear cells (PBMCs) from patients in the United States presenting with mild to moderate COVID-19 symptoms exhibited sexual dimorphism with immune populations, where male patients had more non-classical monocytes and female patients had more robust activated T cell profiles [93•]. In addition, when analyzing immune determinants that correlated with progression to severe disease, men had reduced T cell signatures characteristic of severe disease, and women had elevated viral antigen-specific class-switched antibodies which is predictive of disease protection [93•].

Mouse modeling suggests that estrogen may play a protective role in SARS-CoV-2 infection and COVID-19 disease. Utilizing a mouse-adapted strain of SARS-CoV, the Perlman lab demonstrated that male mice were more susceptible to infection, with significantly increased lung damage and mortality [94••]. In addition, male mice had elevated levels of pro-inflammatory cytokines as well as inflammatory macrophage/monocyte infiltration as compared to female mice [94••]. Importantly, while gonadectomies or treatment with a nonsteroidal anti-androgen had no effect on viral pathogenesis in male mice, gonadectomies and estrogen-receptor antagonist treatment significantly increased viral susceptibility in female mice [94••]. Whether estrogen treatment impacts SARS-CoV infection or disease in male mice remains an open question. While this study demonstrated that estrogen signaling is an important factor in the sex differential susceptibility to SARS-CoV infection, the applicability to SARS-CoV-2 infection is unknown.

An intriguing aspect to the pathogenesis of SARS-CoV and SARS-CoV-2 is that cell tropism relies on the cellular receptor Angiotensin Converting Enzyme 2 (ACE2) and the serine protease TMPRSS2 [95,96,97•,98,99], and expression of both genes exhibits sex differences [100,101]. Both *ACE2* and *TMPRSS2* contain hormone response elements in their promoter regions, and *ACE2* expression is increased by estrogen in human airway epithelial cells [100]. Estrogen-mediated regulation of *ACE2* alone does not account for the male-bias with COVID-19 disease and mortality, as the male bias is observed among older adults, including post-menopausal women who may/may not be on hormone replacement therapy [89]. The *ACE2* gene is X-linked, thus women have the potential to express more ACE2 protein if this gene escapes XCI in specific cell types, as *ACE2* escapes XCI in human fibroblasts [48]. Female-specific increased expression of *ACE2* could facilitate greater SARS-CoV-2 infection (Figure 2), which would likely result in higher cases or greater severity of disease, which is not observed clinically [89]. Recent observations suggest that women express higher levels of *ACE2* in a variety of tissues, and that *ACE2* expression may decrease with age specifically in human male tissues [102]. ACE2 negatively regulates angiotensin II, which promotes lung injury by increasing vascular dysfunction and inflammation [103]. Moreover, angiotensin II can directly induce endothelial apoptosis [104,105] and inhibit endothelial cell proliferation, worsening lung injury [106] and providing an explanatory context for the vascular dysfunction observed in COVID-19 [107]. Thus, elevated ACE2 levels are actually protective, and reduce susceptibility for severe lung injury as well as other vascular-related injuries resulting from SARS-CoV-2 infection [96,103] (Figure 2). An intriguing alternative explanation for elevated *ACE2* expression that may facilitate protection is that *ACE2* is an IFN-stimulated gene [108], thus *ACE2* upregulation may increase viral entry into cells already in a heightened antiviral state [109]. Further examination of the genetic and hormonal contributions that result in differences with *ACE2* and *TMPRSS2* expression in the context of SARS-CoV-2 infection and COVID-19 disease is necessary.

Interplay between viral infection and initiation of autoimmune disease

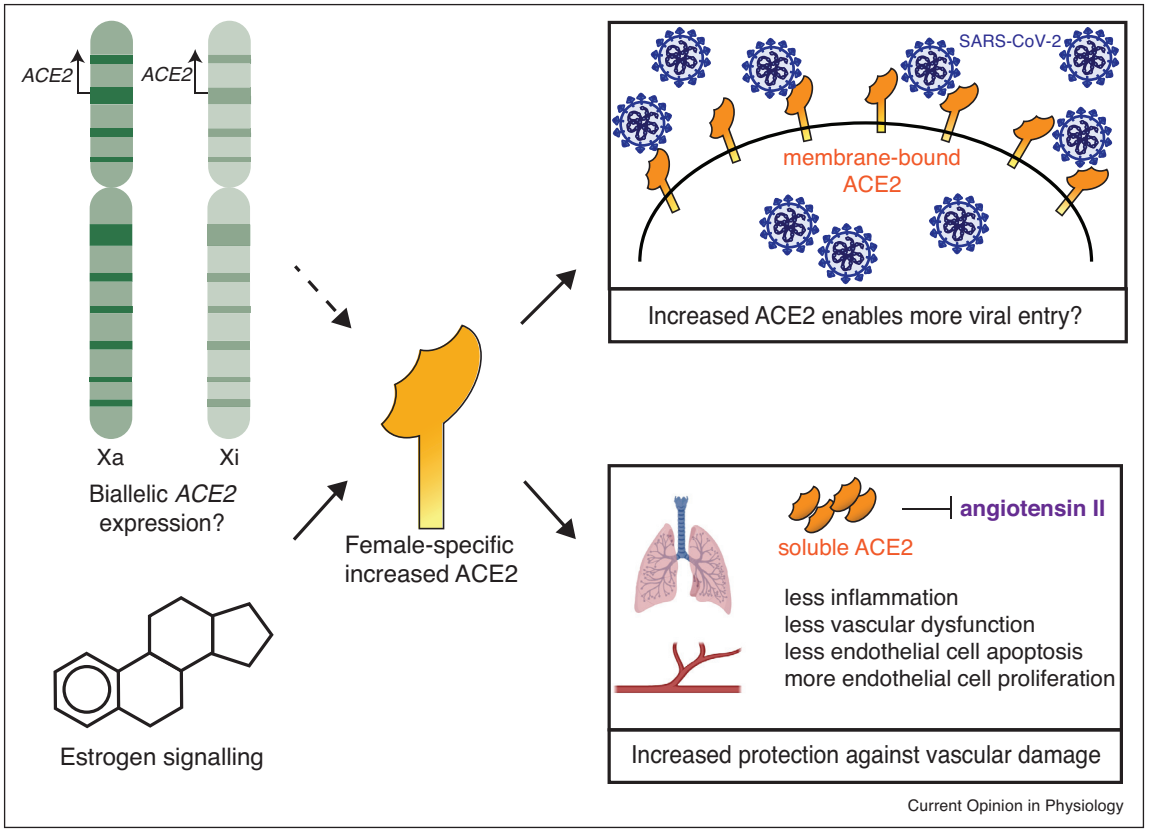
The causes for autoimmune disease are unclear, yet studies suggest that viral infection is strongly correlated with the onset of autoimmunity [110]. Indeed, many different types of viruses have been linked to the development of autoimmunity in humans, including Epstein-Barr Virus, Human Cytomegalovirus, and Human T-Lymphotropic Virus 1 [110–113]. Lymphocytic Choriomeningitis Virus (LCMV) accelerates onset of disease in the classic spontaneous mouse model of lupus, NZB/W F1 mice, which exhibits a female-bias [114]. Viral infection is proposed to drive autoimmunity onset through a variety of mechanisms, where viral-derived T cell-stimulatory molecules (which are structurally similar to self-peptides) result in inflammation and activation of self-reactive T and B cells [110]. Viral infections can often result in abnormal activation of self-reactive T cells, which is a hallmark of autoimmune diseases [115]. Therefore, hormonal and genetic factors that result in more robust female T and B cell activation during viral infections may contribute towards the female-bias with autoimmune disease, and additional research is necessary to elucidate the mechanisms underlying this hypothesis.

Sex differences in autoimmune diseases

About 25 autoimmune disorders have a strong female bias. Sjögren's syndrome, Grave's disease, and Hashimodo's thyroidosis exhibit >90% incidence among women, while Systemic Lupus Erythematosus (SLE), antiphospholipid antibody syndrome, and systemic scleroderma patients are 70–85% female [116,117]. Indeed, autoimmune disease is one of the leading causes of death for women in the United States [116].

One genetic factor that increases the risk for developing SLE or SS is the number of X chromosomes, as 46,XX

Figure 2



Female-biased increased expression of *ACE2* could have different outcomes that alter susceptibility to SARS-CoV-2 and COVID-19 disease severity. Female-specific increases in *ACE2* expression due to genetic and hormonal factors might lead to more viral entry but also leads more active ACE2 and subsequent protection from vascular dysfunction. Legend: ACE2 (orange), SARS-CoV-2 virions (blue).

females and 47,XXY individuals with Klinefelter syndrome display heightened incidence as compared to 46,XY men [116–118]. Individuals with one X chromosome (46,XY males and 45,X Turner syndrome patients) have the lowest risk for developing SLE and SS, while people with more than two X chromosomes (47,XXX Trisomy X syndrome patients) have the highest risk [119–121]. While XCI results in dosage compensation for X-linked genes between males and females, some genes regularly escape silencing [45–51]. It is conceivable that additional immune-linked gene escape can contribute to autoimmune onset and severity, as overexpression of some immunity-related X-linked genes have been observed in SLE patients [122]. Mouse studies have shown that overexpression of *CD40LG* or *BTK* results in autoantibody production and immune complex-mediated glomerulonephritis [123–127], and transgenic overexpression of *Tlr7* in mice can result in various symptoms of autoimmunity, including anti-nucleic acid antibodies, spontaneous lymphocyte activation, and glomerulonephritis [128,129]. Biallelic expression of *Tlr7*, ranging from 10 to 40% of cells, has also been recently observed in human B cells from healthy women, female SLE patients, as well as men with Kleinfelter syndrome [79,130]. Intriguingly, female lupus-prone NZB/W F1 mice also exhibit biallelic expression of *Tlr7* that did not change with disease progression [131••]. A polymorphism in the X-linked gene *CXorf21*, an adaptor for endosomal TLRs including TLR7, is strongly associated with SLE in European populations [132–134]. Whether *CXorf21* escapes XCI is an intriguing open question. These observations suggest that abnormal expression of multiple X-linked genes likely contributes to SLE disease severity.

Lupus disease also affects the localization of epigenetic modifications to the Xi, which may account for X-linked gene expression changes. Circulating T and B cells from both mice and humans display non-canonical features of XCI, as the Xi lacks Xist RNA localization and enrichment of heterochromatic modifications, although *XIST/Xist* is expressed at normal levels [80,135••,136]. *In vitro* stimulation results in accumulation of both XIST RNA and heterochromatic marks on the Xi [80,136]. We

recently found that *in vitro* activated T cells from human SLE patients and both T and B cells from late-stage disease NZB/W F1 mice have dispersed Xist RNA localization patterns [131••,135••]. In addition, T cells from SLE patients had altered expression of X-linked genes, including overexpression of genes involved in metabolism, cell cycle, and proliferation [135••]. Furthermore, B cells from NZB/W F1 mice progressively lost H3K27me3 enrichment on the Xi with increased disease development [131••], suggesting that abnormal XCI is a consequence of lupus disease, and not causal. These findings suggest that perturbed XCI may be responsible for abnormal X-linked gene expression in SLE, and additional work is necessary to determine whether other autoimmune diseases also exhibit perturbed XCI maintenance.

Conclusion

Increasing appreciation of sex as a biological variable will likely to expand our understanding of the underlying genetic and hormonal contributions to observed sex biases for prevalence and disease severity during viral infection and autoimmunity. Here, we highlighted two examples of viral pathogens, influenza and SARS-CoV-2, where clinical observations and mouse models indicate that both X-linked gene expression and hormones contribute to the predominant male bias. The contribution of XCI escape in specific cell types important for flu and SARS-CoV-2 infection are unknown at present, yet will likely reveal important players that contribute to observed sex differences. There are other examples of pathogens, such as *Leishmania* and *Treponema pallidum*, which exhibit sexual dimorphism with infections, cytokine production, and resulting pathologies [137–139]. Yet the genetic contribution of XCI maintenance and XCI escape for these sex differences with pathogen-induced diseases remains unclear. One recent report demonstrated that during *Leishmania* infection, the chemokine receptor *Cxcr3* escapes XCI in T cells [140]. T cells with biallelic *Cxcr3* expression displayed heightened functionality in mice, suggesting that XCI escape could be a significant factor for female-bias with immune responses. While individuals with multiple X chromosomes have higher risk for autoimmune diseases, not all XX individuals will develop an autoimmune disease. Viral infections are correlated with the onset of autoimmunity in women, and more research is necessary to unravel the genetic and hormonal contributions that initiate the pathway for the loss of self-tolerance. Understanding the sex-specific mechanisms of immune responses to pathogens will reveal more effective treatment strategies for pathogen-induced diseases.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Katherine S Forsyth: Writing - original draft. **Montserrat C Anguera:** Writing - review & editing, Conceptualization.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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