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Review article

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The conneXion between sex and immune responses

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Abstract

There are notable sex-based differences in immune responses to pathogens and self-antigens, with female individuals exhibiting increased susceptibility to various autoimmune diseases, and male individuals displaying preferential susceptibility to some viral, bacterial, parasitic and fungal infections. Although sex hormones clearly contribute to sex differences in immune cell composition and function, the presence of two X chromosomes in female individuals suggests that differential gene expression of numerous X chromosome-linked immune-related genes may also influence sex-biased innate and adaptive immune cell function in health and disease. Here, we review the sex differences in immune system composition and function, examining how hormones and genetics influence the immune system. We focus on the genetic and epigenetic contributions responsible for altered X chromosome-linked gene expression, and how this impacts sex-biased immune responses in the context of pathogen infection and systemic autoimmunity.

Sections

Introduction

Sex differences in immune cell composition

Effect of sex hormones on immune cells

X chromosome inactivation

Female-biased systemic autoimmune diseases

Sex differences in responses to pathogens

Sex differences in response to vaccination

Conclusions and perspectives

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Introduction

Biological sex contributes to physiological differences between human male and female individuals that can influence pathogen exposure, recognition and clearance, and replication. Recent studies that investigate how mammals respond to immunogenic challenges have revealed numerous differences between male and female susceptibility to pathogens such as bacteria, fungi, viruses and parasites, probably via sex-biased differences in immune responses (Fig. 1). In general, female individuals have greater innate and adaptive immune responses than male individuals. Female individuals usually clear infections faster than male individuals, and male individuals exhibit increased mortality across all ages, including in pre-term births and infants, after infection[1]. Female individuals often also exhibit stronger serological responses than male individuals following vaccination. Similarly, female mice are more resistant to bacterial and viral infections and generate stronger and longer-lasting immune responses than male mice[2]. This seemingly advantageous female sex bias in the potency of immune responses is juxtaposed by the female-biased proclivity to autoimmunity. Female individuals are at increased risk for some autoimmune diseases, including systemic lupus erythematosus (SLE), Sjögren syndrome and systemic sclerosis (SSc)[3]. However, the diverse molecular mechanism underlying these sex-biased immune responses remains incompletely understood, and clarifying their origin may help to provide new insights into disease pathogenesis.

Immune responses can be affected by the X and Y chromosomes, gonadal sex hormones and variations in levels of sex hormones over time. Sex hormones, specifically androgens and oestrogens, can have either pro-inflammatory or anti-inflammatory effects, and many genes that are involved in immune regulation are regulated via the binding of these hormones to nuclear hormone receptors (Box 1). Sex chromosomes provide the genetic and epigenetic foundations for altered gene expression in a heritable, sex-specific manner in immune cells. The X chromosome contains about 50 genes with immune functions, including some that are important for immune cell identity (notably, *FOXP3*), cellular activation and intracellular signalling (*CD40LG*, *TLR7*, *IRAK1*, *IL13RA1/2*, *NEMO* (also known as *IKBKG*), *TASL* (also known as *CXorf21*) and *IL9R*), leukocyte trafficking (*CD99* and *CXCR3*), immune cell differentiation and proliferation (*IL2RG* and *BTK*) and cellular metabolism (*OGT*). Thus, the X chromosome is probably an important contributor to the molecular basis of female-biased antimicrobial responses and autoimmunity.

In this Review, we analyse the current literature on sex differences in immune system composition and function, and examine how sex hormones and the genetics of the X chromosome affect immunity. In particular, we examine genetic and epigenetic contributions to altered X-linked gene expression and how these impact female-biased autoimmune disorders. We also highlight examples of altered X-linked gene expression that influence pathogen susceptibility, infectious disease progression and responses to vaccination.

Sex differences in immune cell composition

Sex-biased differences in innate, humoral and cellular immune responses that have been observed in humans are partially due to sex differences in immune cell composition. For example, flow cytometric immunophenotyping of healthy volunteers in Europe and Asia have consistently identified female-specific increases in the number of $CD4^+$ T cells, specifically naive cells, which may reflect enhanced thymic function[4,5]. A recent whole-blood transcriptional profiling study of multiple international and intercontinental cohorts of healthy individuals similarly identified a higher proportion of $CD4^+$ T cells in female individuals[6]. In addition, female individuals have more circulating $CD19^+$ B cells, plasma cells, regulatory T (T_{reg}) cells (defined by $CD25^{hi}CD127^{lo}$) and both naive $CD8^+$ and mucosa-associated invariant T cells than male individuals[4,7–10]. Conversely, female individuals have lower proportions of both $CD14^+$ (classical) and $CD16^+$ (non-classical) monocytes, a generally lower proportion of myeloid cells[6,8] and fewer natural killer (NK) cells[4,7,8,11] than male individuals. Differences in humoral immunity have also been observed between the sexes, with female individuals producing higher quantities of IgM antibodies[12].

Similarly, sex differences in immune cell composition have been observed across mouse strains[13,14]. For example, female C57BL/6 mice have increased proportions of B and T cells in the peripheral blood compared with their male counterparts[14]. Sex differences in the immune cell composition of mice have also been identified in the specific tissues, including pleural and peritoneal cavities[15], as well as the spleen[15,16]. Mesenteric tissues from female animals express a higher level of genes encoding chemokines and chemokine receptors, which can enhance the recruitment of both innate and adaptive immune cells. A recent study has found that male mice have higher numbers of splenic NK cells, yet these have reduced effector functions due to lower expression of the X-linked gene *Kdm6a* (also known as *Utx*), a histone lysine demethylase, compared with NK cells from female mice[17]. Curiously, sex hormones do not significantly influence NK cell number or effector function, as a NK cell-specific deletion of *Utx* in female mice increases their frequency and reduces their effector function. This was found to be due to aberrant upregulation of BCL2, an anti-apoptotic factor, and downregulation of IFNγ[17]. The identification of additional X-linked genes that can affect the frequencies of both innate and adaptive immune cells may help to further clarify the basis of the observed differences in immune cell composition between the sexes.

Effect of sex hormones on immune cells

Sex differences in immune cell function arise from differential gene expression in immune cell subsets of male and female individuals, and sex hormones can contribute towards these differences. Transcriptional profiling of human innate and adaptive immune cells – including monocytes, naive B cells and various T cell subsets – identified about 1,875 transcripts that exhibit sex-biased expression, the majority of which were autosomal[18]. Most of these transcripts only showed sex-biased expression in one type of immune cell, highlighting the cellular specificity of sex-specific gene expression and the broad effect of sex hormones and *trans*-acting sex chromosomal elements on the observed differences in gene expression[18]. Oestrogens can have either pro-inflammatory or anti-inflammatory functions (Box 1), depending on the local hormone concentration and the immune cell examined. For example, transcription of *AICDA*, which encodes AID, a cytidine deaminase that is important for somatic hypermutation and class-switch recombination in B cells, is regulated by binding of the oestrogen–oestrogen receptor-α (ERα) complex to the promoter of *AICDA*. Of note, decreased or increased AID expression has each been associated with the development of systemic autoimmunity, suggesting that the regulation of AID is important for maintaining immune tolerance[19]. Oestrogens also increase the expression of endosomal Toll-like receptor 3 (TLR3), TLR7 and TLR9, which are important regulators of type I IFNs[20]. Co-culture of B cells with oestrogen-treated $CD4^+$ T cells from patients with SLE resulted in increased antibody production compared with in vitro co-culture with T cells from healthy controls[21].

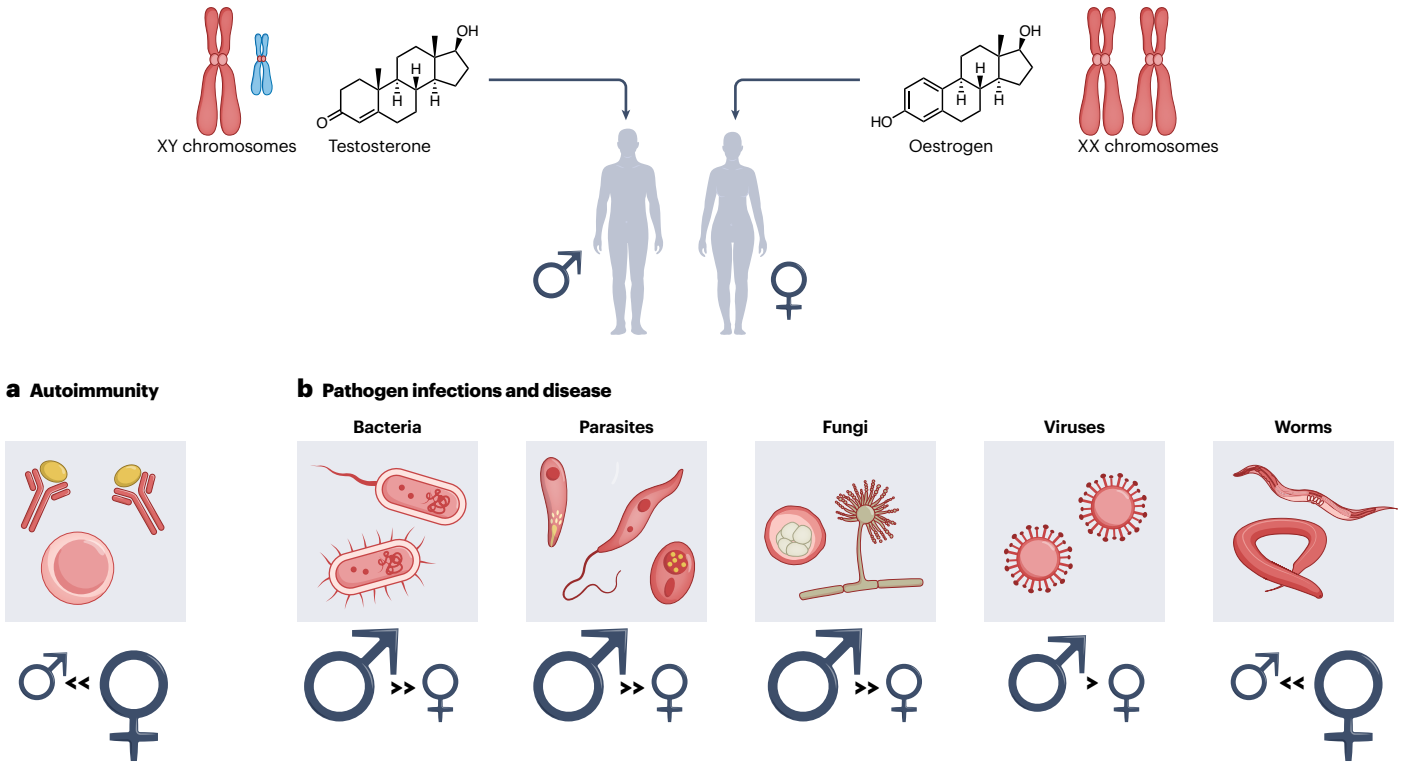

Fig. 1 | Sex differences with pathogen infections and autoimmune disease. **a**,**b**, The sex chromosomes and the sex hormones in biological male (XY) and female (XX) individuals are responsible for the observed sex differences in the susceptibility to systemic autoimmune diseases (**a**) and infection by various pathogens (**b**). The overall sex bias (with regards to susceptibility to infection and disease severity) that is associated with a particular class of pathogen infection (and associated disease) is indicated (the size of the symbol reflects the degree of sex bias). Systemic autoimmune diseases such as systemic lupus erythematosus, Sjögren syndrome and systemic sclerosis are strongly biased towards female individuals.

Type I IFN production and antiviral responses downstream of TLR3, TLR7 and TLR9 are regulated by the ubiquitin ligase TRIM21, which is also upregulated by oestrogen. TRIM21 expression also promotes the secretion of IL-23, which promotes the differentiation of T helper 17 (T_H17) cells. This pathway is integral to the pathogenesis of several systemic autoimmune diseases, including SLE, SSc, Sjögren syndrome and rheumatoid arthritis[22]. The female bias for enhanced type I IFN signalling has also been observed in human plasmacytoid dendritic cells (pDCs), which produce IFNα following TLR stimulation. Human female pDCs treated with TLR7 and TLR8 agonists, but not TLR9 agonists, produce more IFNα than pDCs from male individuals, and display higher transcription of the IFN-stimulated gene *IRF5* than pDCs from male individuals[23–25]. Although a sex bias with regards to type I IFN production in response to TLR9 stimulation was not observed in the original study, this might have been due to the type of CpG used in the original study[23], as sex-biased type I IFN production by pDCs in response to TLR9 stimulation has recently been reported[26,27]. It is likely that both sex chromosomal and sex hormonal mechanisms contribute towards this bias given that *TLR7* and *TLR8* are X-linked, and *IRF5* transcription is regulated by oestrogen receptor 1 (ESR1)[28].

Sex differences in immune functions, particularly in the context of microbial infections, also reflect differences in the cellular expression of pattern recognition receptors. For example, peritoneal macrophages from female rodents express higher levels of *Tlr2*, *Tlr3* and *Tlr4*, and exhibit enhanced phagocytosis and NADPH-mediated bacterial elimination compared with their male counterparts[15]. Accordingly, peritoneal-derived macrophages from female mice also show increased expression of IFN-stimulated genes, and in a mouse model of sepsis, female mice became less sick and had fewer recoverable live bacteria in their circulation than male mice[29,30]. Previous ovariectomy equalized responses between male and female mice in this model, which indicates a role for sex hormones in shaping the phenotype of tissue-resident immune cells. Bone marrow-derived macrophages from female mice have been shown to express higher levels of *Tlr8* than male mice[31], and peripheral blood mononuclear cells from human female individuals express more *TLR7* than male individuals[32]. Vaccination or viral challenge increases the expression of genes encoding TLR pathway components and pro-inflammatory factors in female peripheral blood mononuclear cells from humans and rats, and it has been suggested that this is due to oestrogen binding at sex hormone receptor elements in the promoter regions of these genes[33]. However, neutrophils from male humans have higher expression of *TLR4* than their female counterparts, and the difference in expression increases following activation with lipopolysaccharide, which results in higher pro-inflammatory cytokine production in male individuals than in than their female counterparts and may contribute towards the male bias in susceptibility to endotoxic shock[34]. The molecular basis for the increased expression of TLR4 in human neutrophils is not entirely clear, as androgens are generally considered to have an anti-inflammatory role (Box 1). Furthermore, androgen treatment of male mice has been shown to decrease *Tlr4* expression in

Box 1

Sex hormones and inflammation

The oestrogens oestrone, 17β-oestradiol and oestriol, as well as progesterone and the androgens testosterone and dihydrotestosterone are the predominant gonadal sex hormones. The sex hormone receptors oestrogen receptor-α (ERα) and ERβ, progesterone receptor and androgen receptor are hormone-activated transcription factors that bind to hormone response elements and regulate gene expression. Oestrogen receptors, progesterone receptors and androgen receptors are expressed by various immune cells such as T cells, B cells, dendritic cells, monocytes and macrophages, natural killer cells, type 2 innate lymphoid cells and granulocytes, as well as cells at the interface of skin and mucosal barrier sites, and thymic epithelial cells[174]. Sex-biased gene expression can result from sex-specific differences in steroid abundance. For example, numerous oestrogen receptor response elements in the *IFNG* promoter allow for increased downstream gene expression when 17β-oestradiol levels are increased, thereby contributing to female-biased increased production of IFNγ[175].

Oestrogens can exhibit either anti-inflammatory (at high concentrations) or pro-inflammatory (at low concentrations) effects[176]. During pregnancy or periovulation when oestrogen concentrations are high, oestrogens exert inhibitory effects on T cells (specifically T helper 1 (T_H1) and T_H17-polarized cells), M1-polarized macrophages, dendritic cells, neutrophils and microglia through NF-κB inhibition, and can also increase regulatory T cell function. ERα in T cells can promote T cell activation-induced apoptosis and downregulate *Foxp3* expression[177], and can also suppress T follicular helper cell development[178]. Oestrogens can also signal through ERβ in regulatory T cells in the intestine to regulate immune suppression[179]. Both ERα and ERβ in $CD4^+$ T cells are important for ligand-mediated suppression of autoimmunity of the central nervous system (particularly in the context of multiple sclerosis) by targeting pathogenic T_H17 cells[180,181]. ERβ is also required for regulatory T cells to regulate macrophage pro-inflammatory responses and facilitate the resolution of lung inflammation resulting from pneumonia[182].

The effect of oestrogen on the immune response is also influenced by the immune microenvironment. In animal models of lupus-like disease, oestrogens can be pro-inflammatory or anti-inflammatory, depending on the nature of the pro-inflammatory milieu that is responsible for the observed lupus-like phenotypes. In lupus-prone F_1 hybrid New Zealand black–white (NZB/W) mice, deletion of the gene encoding ERα in B cells reduced autoantibody production and nephritis[183]. In the MRL/lpr mouse model of spontaneous lupus-like disease, administration of exogenous oestrogen resulted in accelerated immune-complex glomerulonephritis, reflecting a pro-inflammatory effect via B cell activation and autoantibody production; however, T cell-mediated disease, including periarticular inflammation, focal sialadenitis and renal vasculitis, was significantly reduced[176,184]. Inflammatory cytokines including TNF, IL-1 and IL-6 can stimulate the activity of aromatase, an enzyme required for oestrogen biosynthesis. Thus, the inflammatory milieu may confer contextual effects by altering the local concentration of oestrogen[185]. The concentration-dependent and context-dependent effects of oestrogen are also apparent in examples of systemic autoimmune disease. In rheumatoid arthritis and multiple sclerosis, disease activity often spontaneously decreases during pregnancy, a phenomenon that has been attributed to the anti-inflammatory effects of high concentrations of oestrogen[180,181,186]. Nevertheless, the cumulative effects of local oestrogen abundance are not so straightforward, as disease activity in systemic lupus erythematosus often spontaneously increases during pregnancy[187]. These observations highlight the complex interactions between sex hormones, immune cells, immune tolerance and the developing fetus.

Androgens have anti-inflammatory effects on immune responses in vivo and in vitro, through diverse mechanisms. In the context of their effects on the innate immune system, they have been shown to modify signal transduction through pattern recognition receptors, suppressing the genes encoding both NF-κB and TLR4 expression[188]. Androgens also reduce the secretion of pro-inflammatory cytokines such as TNF, IL-1β, and IL-6 from monocytes and macrophages, and IL-33 production from mast cells[38,189,190]. Androgens impact adaptive immune responses and can inhibit both humoral and cellular immune responses by reducing lymphocyte proliferation and decreasing immunoglobulin and cytokine production[36,38,189,190]. Androgens have inhibitory effects on B cell lymphopoiesis and specifically affect the production of B cell precursors, which express androgen receptor, whereas mature B cells, which lack this receptor, are not directly impacted by androgens[38]. $CD4^+$ T cells from female mice treated with dihydrotestosterone increase IL-10 production compared with untreated female animals[37].

macrophages, suppress the invasion and colonization of uropathogenic *Escherichia coli* through inhibition of the JAK–STAT signalling pathway, and decreases production of IL-1β, IL-6 and IL-8 (ref. 35).

The anti-inflammatory effects of androgens on both humoral and cellular immune responses are also the result of diverse mechanisms, including their inhibitory activity on T and B cell proliferation, which leads to a decrease in immunoglobulin and cytokine production[36]. Female mice treated with the androgen dihydrotestosterone produce more IL-10 and less IL-12 than untreated female animals, resulting from increased androgen receptor signalling in $CD4^+$ T cells[37]. Androgens also have an inhibitory effect on B cell lymphopoiesis and specifically impact B cell progenitors, which express *AR*, whereas mature B cells lacking this receptor are not affected[38]. Unlike oestrogen, which upregulates the expression of *BAFF*, androgens inhibit *BAFF* expression[39]. Thus, androgens block BAFF-dependent B cell clonal expansion and class-switch recombination, and downregulate germinal centre responses through B cell extrinsic mechanisms[40]. Androgens also increase the expression of *PTPN1*, a tyrosine phosphatase that affects cellular proliferation,

differentiation, mitosis and immune cell function. Here, androgen receptor signalling prevents the development of T_H1 cells by upregulating the expression of *Ptpn1*, which encodes the phosphatase PTPN1. PTPN1 dephosphorylates and inhibits the kinases TYK2 and JAK2 upstream of STAT4, thereby blocking JAK–STAT signalling pathways important for T_H1-mediated immune responses and the production of IL-12 and IFNγ[41].

Androgens also have an important role in central tolerance by regulating the thymic expression of *AIRE*, a transcription factor that is important for the elimination of self-reactive T cells. Androgens recruit androgen receptors to the *AIRE* promoter and enhance its transcription in male mice and humans, whereas oestrogens inhibit *AIRE* expression[42,43]. Androgens can also affect the expression of *FOXP3*, which encodes a transcription factor that has a central role in the development of $CD4^+$ T_{reg} cells. Evidence exists that androgen treatment in humans can increase *FOXP3* expression in female T_{reg} cells during the ovulation phase of the menstrual cycle in female individuals, without any effect on T_{reg} cells from male individuals[44].

X chromosome inactivation

Female mammals regulate X-linked gene expression using X chromosome inactivation (XCI) (Box 2), in which one X chromosome (the inactive X chromosome (Xi)) is transcriptionally silenced during development, and various epigenetic modifications ensure that this silencing is maintained with each cell division and persists into adulthood. These epigenetic modifications, as well as the long non-coding RNA Xist, are enriched across the Xi and function to maintain transcriptional repression of most, but not all, of the genes on the Xi. Xist expression is required to form the Xi, and loss of Xist in early development is lethal due to the requirement for an appropriate dosage of X-linked genes in both embryonic and placental development[45]. Deletion or gene silencing of Xist reduces, and often eliminates, the enrichment of heterochromatic modifications from the Xi[46] and results in reactivation of some X-linked genes on the Xi, depending on the cell type and timing relative to XCI initiation. Mouse models with conditional tissue-specific Xist deletion in post-XCI somatic cells yield variable phenotypes, including myeloproliferative neoplasia (in response to Xist deletion in haematopoietic stem cells), increased polyp formation (in response to Xist deletion in intestinal cells following azoxymethane/dextran sulfate treatment) or even lack of a perceptible phenotype (in response to Xist deletion in neurons, B cells, epithelial cells and intestinal cells), in which animals are viable and have normal lifespans[47–49]. Collectively, these studies highlight three fundamental observations: (1) the identity and number of X-linked genes exhibiting reactivation upon Xist deletion varies by cell type; (2) X-linked dosage imbalances are, in fact, variably tolerated in a

Box 2

XCI and 'escape' from XCI

Female eutherian mammals use X chromosome inactivation (XCI) for dosage compensation of X-linked genes between the sexes[191]. XCI is initiated during early embryonic development, during which one X chromosome is randomly selected for transcriptional silencing. The process begins with upregulation of the long non-coding RNA Xist, which spreads across the future inactive X chromosome (Xi) in *cis* together with Polycomb repressive complexes (PRCs)[192–197]. X-linked gene promoters and enhancers rapidly lose active histone acetylation marks concurrently with transcriptional repression[194–196]. The repressive histone modifications H2AK119Ub and H3K27me3 are deposited on the Xi by PRC1 and PRC2, respectively, and the histone variant macroH2A becomes enriched[194,195,198,199], together creating a heterochromatic environment across the Xi. As a final layer of regulation to maintain transcriptional repression of the Xi, DNA methylation becomes enriched at promoters and enhancers[200–204]. Thus, in most somatic cells, transcriptional silencing of the Xi is maintained with each cell division through Xi enrichment of Xist RNA, heterochromatic histone marks and DNA methylation, some of which can be visualized cytologically using RNA fluorescence in situ hybridization and immunofluorescence.

Although most genes on the Xi are silenced, approximately 15–23% of X-linked genes in humans and 3–7% of X-linked genes in mice are transcribed from the Xi and 'escape' XCI, either constitutively or in a cell-type-specific manner[73,205–207]. The promoter regions of XCI escape genes lack Xist RNA enrichment[194,195,197], heterochromatic histone marks[194,196] and DNA methylation[203,204], and are enriched for RNA polymerase II, DNAse I hypersensitivity sites and CTCF occupancy, which collectively allow for gene expression from a highly heterochromatic environment. X-linked genes with a Y-linked counterpart (XY gene pairs) commonly escape XCI silencing and are expressed from the active X chromosome (Xa) and Xi; however, transcript levels from the Xi are typically less than levels from the Xa[208]. Importantly, X-linked genes lacking a Y-linked homologue can also escape XCI. Both the magnitude and the extent of XCI gene escape vary considerably by cell type, as well as between individual cells and among individuals. In addition, some escape genes display sex-biased expression[32,73,209,210]. Of the approximately 54 immunity-related genes located on the mouse and human X chromosomes, 20.4% are expressed more highly in females and 16.7% are expressed more highly in males[211]. However, these values are probably underestimates due to the methodological challenges associated with measuring transcription specifically from the Xi. Because XCI is random and because expression from the Xi is typically less than that of the Xa, the identification of XCI escape genes requires single-cell approaches with high-sequencing depth or bulk-cell analyses using F_1 hybrid mice with skewed XCI[72,206,212]. Nevertheless, ongoing efforts to identify additional escape genes are likely to provide important insights into sex-biased immune responses — to date, several pro-inflammatory genes have been found to aberrantly escape XCI in systemic lupus erythematosus and other autoimmune diseases, suggesting a key role for the transcription of X-linked genes from the Xi in the context of female-biased immunological processes (see Table 1).

cell-type-specific manner; and (3) inflammatory stress may exacerbate Xi reactivation.

Female mammals are mosaic for the X chromosome, as each individual female cell will contain an Xi that is either maternal or paternal in origin. Most somatic cells examined to date have similar epigenetic features enriched on the Xi, including Xist RNA, heterochromatic histone modifications and histone variant macroH2A, which can be visualized cytologically[50–53]. However, immune cells lack some hallmark epigenetic features of canonical XCI maintenance. Specifically, splenic B and T cells from mice and circulating lymphocytes from humans lack cytologically visible Xist RNA and heterochromatic mark enrichment at the Xi, despite the lack of any significant changes with regards to Xist transcription[54–57]. Thus, Xist transcription and its localization to the Xi are genetically independent processes. In vitro activation of B and T cells stimulates the return of Xist RNA and heterochromatic modifications to the Xi, before the first cell division[54–56,58]. This dynamic localization of Xist RNA and heterochromatic marks to the Xi is also observed during lymphocyte development, as Xist RNA is lost from the Xi after the common lymphoid progenitor stage, at the pro-B cell and DN1 thymocyte stages, whereas Xist transcription remains constant[55,56].

Surprisingly, non-canonical XCI maintenance is a feature of both adaptive and innate immune cells. Xist RNA exhibits substantial diversity in the robustness of its relocalization to the Xi depending on the type of immune cell. Neutrophils and pDCs lack detectable Xist RNA transcripts at the Xi; by contrast, NK cells exhibit pinpoints of Xist RNA signals detected by RNA fluorescence in situ hybridization within the Xi territory[59]. Mouse bone marrow-derived macrophages stimulated with either lipopolysaccharide or CpG have dispersed Xist RNA patterns, and about half of the nuclei have a trimethylated histone (H3K27me3) focus that colocalizes with Xist. In vitro stimulation of mouse pDCs with either CpG or a TLR7 agonist did not result in detectable Xist RNA localization at the Xi, despite persistent Xist transcription, and both resting and in vitro-activated pDCs have few H3K27me3 foci[59].

The paucity of epigenetic features on the Xi in many immune cells, including lymphocyte progenitors, supports the hypothesis that the chromatin of the Xi in immune cells is more euchromatic than in fibroblasts, and therefore prone to aberrant reactivation at certain loci. The facultative nature of the chromatin of the Xi may facilitate the increased expression of pro-inflammatory X-linked genes in response to pathogen infections, which would provide an advantage to female individuals, and yet simultaneously disrupt the balance of self-tolerance, resulting in the development of autoimmune diseases predominantly in female individuals (Fig. 1).

Table 1 | Prevalence and incidence of some female-biased systemic autoimmune diseases in the USA

Autoimmune disease	Prevalence in the USA (per 100,000 people)	Incidence in the USA (per 100,000 person-years)	Sex bias (% of affected individuals who are female)
Systemic lupus erythematosus	5–241 (ref. 131)	1.0 – 23.2 (ref. 131)	66–93 (ref. 131) 83.71 (ref. 60)
Sjögren syndrome	22–103 (ref. 132)	3.9 (ref. 133)	90.54 (ref. 60) 96.2 (ref. 133)
Systemic sclerosis (scleroderma)	27.6 (ref. 134)	1.93 (ref. 134)	83.7 (ref. 134) 75–93.5 (ref. 135) 83.80 (ref. 60)
Inflammatory myopathies: polymyositis and dermatomyositis	6.3 (ref. 67)	0.116–0.6 (ref. 67)	65.08 (ref. 60) 60–75 (ref. 67)
Rheumatoid arthritis	1,070 (ref. 136) 1,000 (ref. 67)	75.3 (ref. 136)	73.4 (ref. 136)

Female-biased systemic autoimmune diseases

Autoimmune diseases predominantly affect female individuals, to the extent that 80% of all individuals with autoimmune disease are female[60–62]. For some autoimmune diseases, such as multiple sclerosis, this sex bias is modest (around 75% female)[63], whereas for other autoimmune diseases, such as primary biliary cirrhosis, the sex bias is strong (roughly 90% female)[64]. The female sex bias for some autoimmune diseases has increased over time. For example, in the mid-twentieth century, multiple sclerosis exhibited an equal prevalence between the sexes; by the 1980s, the sex ratio was 2:1 (female:male); currently, the sex ratio is 3:1 (refs. 63,65). The observed increase in the female bias of disease probably reflects advances in the medical classification and diagnosis of these diseases but could also reflect environmental changes. Although the female bias for many autoimmune diseases is quite high, the incidence and prevalence of autoimmune diseases can vary. Some diseases, such as rheumatoid arthritis, impact large numbers of people (US prevalence of approximately 1,000 per 100,000 people[66]), and some, such as inflammatory myopathies, are much rarer (US prevalence of 6.3 per 100,000 people[67]) (Table 1). Importantly, there are also sex differences in disease severity, specifically with respect to the degree of organ involvement and symptom burden and/or disability. Male patients with multiple sclerosis, SLE and SSc typically exhibit greater disease severity[68,69], and male patients with SLE are more likely to exhibit renal and cardiovascular comorbidities[70]. However, this male-biased increase in autoimmune disease severity is not universal; for example, in rheumatoid arthritis, biological sex does not correlate with articular disease severity[71]. Translational studies that systematically examined the relationship between biological sex and both disease manifestations and disease severity are necessary to better understand how biological sex may impact the phenotype, pathogenesis and natural history of disease.

Dosage imbalances of some X-linked genes are detrimental for cell function and can result in features of systemic autoimmune disease[64]. Because XCI escape (Box 2) is variable across cell types, it is likely that some of these X-linked genes are biallelically expressed in specific cell types in healthy individuals, and therefore exhibit sex-biased expression[72]. The X chromosome contains many genes that function, either directly or indirectly, in immune processes[1]. It is therefore likely that many immunity-related X-linked genes are dosage sensitive and therefore subject to XCI silencing in healthy immune cells, and become aberrantly reactivated from the Xi in response to infection or with disease[58,73]; however, direct evidence from specific cell types in mice and human samples is lacking. It is also unclear whether any of these genes can become reactivated from the Xi in response to antigen-mediated stimulation or pathogen infection, or as a result of chronic inflammation during autoimmunity. Aberrant XCI maintenance, in which XIST RNA and specific heterochromatic histone modifications (H3K27me3 and H2AK119-ubiquitin) are missing from the Xi, has been observed in circulating lymphocytes from female paediatric and adult patients with SLE and also in various mouse models of spontaneous lupus-like disease, which exhibit a female bias[56,58,74,75]. We have proposed the hypothesis that systemic

autoimmune diseases, such as SLE, result in reduced enrichment of epigenetic modifications across the Xi, which permits the reactivation of genes related to immune functions, resulting in their aberrantly increased expression in lymphocytes[54,56,64,74] (Fig. 2). Additional work is necessary to determine whether perturbed XCI maintenance is also a feature of other autoimmune diseases that predominantly affect female individuals.

In addition to female individuals with two X chromosomes, individuals with Klinefelter syndrome (XXY) and with polysomy X (XXX) have increased susceptibility to autoimmune diseases such as SLE, Sjögren syndrome, SSc, and polymyositis and dermatomyositis[76,77] (Fig. 3). The association between female-biased autoimmunity and X chromosome dosage may also apply more broadly to other autoimmune diseases pending additional karyotype-stratified case–control studies. The contribution of multiple X chromosomes to systemic autoimmune disease risk (independent of sex hormones) is supported by mouse models using the 'four core genotypes': (1) ovary-bearing XX mice, (2) ovary-bearing XY mice deficient in the Y-linked *Sry* gene that is required for sex determination and male gonad formation (XY *Sry*$^{-}$ mice), (3) testes-bearing XY*Sry* and (4) testes-bearing XX*Sry* transgenic mice[78]. In mouse models of SLE (pristane-induced) and multiple sclerosis (experimental autoimmune encephalomyelitis), XX and XX*Sry* animals experience an accelerated onset and increased disease severity[79], reflecting genetic and epigenetic contributions from the X chromosome; however, the causal genes responsible for this phenotype are unknown. Ovariectomized female F_1 hybrid New Zealand black–white (NZB/W) mice spontaneously develop SLE-like disease in the absence of female sex hormones (Box 3). Injection of haematopoietic stem cells or fetal liver cells from these mice into hormonally intact lethally irradiated NZB/W F_1 male mice results in the development of lupus-like disease with increased numbers of germinal centre B cells, memory B cells and plasma cells in all male recipients[80]. This indicates that X-linked genes expressed in female (XX) immune cells, independent of previous exposure to female sex hormones, accelerate SLE-like disease onset in male animals predisposed to develop autoimmune disease, highlighting the significance of genetic contributions from the X chromosome in SLE. Collectively, these studies demonstrate the contributions of the X chromosome to female-biased autoimmunity and highlight the necessity for additional studies to determine which X-linked genes drive sex bias in autoimmunity via their expression from the Xi.

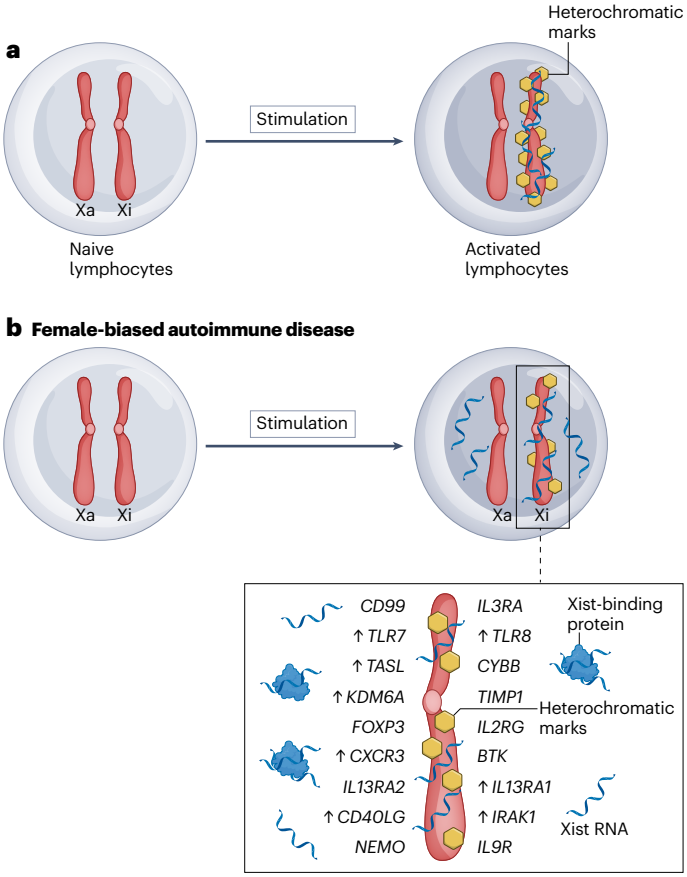

Fig. 2 | Impairments in dynamic XCI maintenance result in aberrant overexpression of X-linked genes in female-biased autoimmune diseases. **a**, The inactive X chromosome (Xi) in naive lymphocytes lacks the cytological enrichment of Xist RNA and heterochromatic marks such as H3K27me3 and H2AK119-ubiquitin typically observed in other somatic cell types, and these modifications return to the Xi in activated cells. **b**, Activated lymphocytes from female patients with systemic autoimmune diseases such as systemic lupus erythematosus have dispersed Xist RNA and heterochromatic marks that are not cytologically enriched on the Xi, and increased expression of some X-linked genes. Prevention of Xist RNA tethering to the Xi reduces enrichment of histone heterochromatic marks on this chromosome, and persistent absence of these epigenetic modifications across multiple cell divisions may increase abnormal overexpression across the Xi. Xa, active X chromosome; XCI, X chromosome inactivation.

Dosage sensitivity of X-linked immune-related genes

A number of genetic immunodeficiencies are X-linked, indicating the importance of appropriate X-linked gene dosage for immune function and health[81]. Accordingly, X-linked genes that are aberrantly expressed in immune cells of patients with female-biased autoimmune disease suggest that perturbations in the regulation of XCI contributes to this bias. Abnormal X-linked gene expression has been observed in both adaptive and innate immune cells from patients with autoimmune rheumatic diseases, including SLE, Sjögren syndrome and SSc[82] (Table 2). Interestingly, transgenic mouse models overexpressing some X-linked genes related to immune function display features of autoimmune disease (Table 2). For example, the X-linked gene *CD40LG*, which encodes a receptor for CD40, is primarily expressed by activated CD4^{+} T cells and binds to CD40 expressed by dendritic cells, B cells and endothelial cells. Compared with healthy female individuals, *CD40LG* is aberrantly overexpressed in T and B cells from female patients with SLE[83,84] and SSc[85]. Although the biological significance of this overexpression in these patients is not well established, transgenic overexpression of *Cd40lg* in mice results in autoimmune disease, with increased IgG antibodies, chronic inflammation, glomerulonephritis, thymic atrophy and increased lethality[86].

CXCR3 is an X-linked gene encoding an inducible chemokine receptor that affects adaptive immune responses by regulating T_H1 cells and T cell trafficking. *CXCR3* is overexpressed in circulating CD4^{+} T cells of female patients with SLE, and the proportion of CXCR3^{+}CD4^{+} T cells is increased in the urine and kidneys from individuals with lupus nephritis[84,87]. Individuals with Sjögren syndrome have increased numbers of infiltrating CXCR3^{+}CD3^{+} T cells in salivary

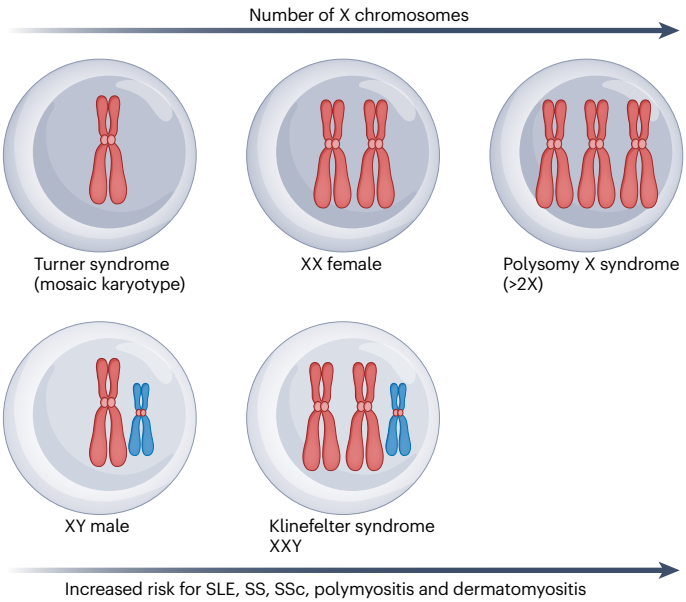

Fig. 3 | Number of X chromosomes and risk for autoimmune disease. An increased number of X chromosomes in healthy female individuals or in individuals with Klinefelter syndrome (XXY) or polysomy X syndrome (XXX) is associated with a higher risk for the female-biased autoimmune diseases systemic lupus erythematosus (SLE), Sjögren syndrome (SS), systemic sclerosis (scleroderma) (SSc), and polymyositis and dermatomyositis.

gland tissue and also show enhanced expression of the CXCR3 ligands CXCL9 and CXCL10 (ref. 88).

The X-linked gene *BTK* encodes a kinase that is associated with the B cell receptor and its activation induces a signalling cascade that is required for B cell proliferation, activation and survival. Patients with SLE (samples were not distinguished by sex) with active lupus nephritis overexpress *BTK* in peripheral blood mononuclear cells[89], and *Btk* overexpression in mice promotes the development of germinal centre B cells and leads to the onset of systemic autoimmune disease as evidenced by increased positive antinuclear autoantibody staining as well as immune complex deposition in the kidneys[90]. Decreasing the activity of BTK with small-molecule inhibitors in both a spontaneous (NZB/W F_1 mice) and an inducible mouse model of SLE reduces lupus-like disease phenotypes[91].

FOXP3 encodes an X-linked transcription factor that is important for the differentiation and maintenance of T_{reg} cells. T_{reg} cells from patients with SLE or SSc express lower levels of FOXP3, which probably compromises their function, and although there are conflicting reports regarding the abundance of T_{reg} cells in the peripheral blood of patients with SLE and SSc, available evidence suggests that T_{reg} cells may be dysfunctional in these diseases[92–94]. T_{reg} cells in the skin of individuals with SSc exhibit abnormal production of T_H2 cell cytokines, suggesting localized tissue-specific T_{reg} cell dysfunction that may contribute towards fibrosis[95]. Overexpression of *Foxp3* is protective against renal dysfunction in a mouse model of accelerated crescentic glomerulonephritis due to increased T_{reg} cell number and function, which systemically suppress T_H1, T_H2 and T_H17 responses[96].

The X-linked gene *TLR7* encodes an endosomal pattern recognition receptor that induces type I IFN production and therefore activates IFN signature genes. Increased *TLR7* expression was observed among female paediatric patients with SLE[97]; this may be partly due to its ability to escape XCI and exhibit biallelic expression in immune cells, such as B cells[32]. Biallelic expression of *TLR7* in female human B cells increases cellular responsiveness to TLR7 ligands and increases class switching compared with monoallelic *TLR7*-expressing B cells[32]. The resulting increase in TLR7 signalling is probably biologically significant. Recently, a gain-of-function mutation in *TLR7* was identified in a young female individual with SLE. Introduction of this mutation into C57BL/6 mice results in the spontaneous development of lupus-like disease[98]. Mouse models with two or more copies of *Tlr7*, including the BXSB/*Yaa* strain (Box 3), have increased autoantibody production, autoreactive lymphocytes and glomerulonephritis[99,100]. *TLR7* exhibits variable escape from XCI in human immune cells and B cells from female NZB/W F_1 mice[75], suggesting that its aberrant overexpression resulting from XCI escape is a mechanism that may contribute towards female-biased autoimmune disease. *TASL*, an X-linked adaptor protein critical for TLR7 signalling, is aberrantly overexpressed in lymphoblastoid cell lines from patients with SLE compared with healthy controls and may escape XCI in immune cells[101]. Thus, abnormal overexpression of *TLR7* and *TASL* due to potential perturbations with XCI escape could represent a mechanism that contributes towards increased IFN signalling in female individuals with autoimmune disease.

The retrospective identification of additional disease-related genes abnormally expressed from the X chromosome is challenged by the frequent omission of sex chromosome reads in microarray and transcriptional profiling datasets when normalizing samples between the sexes. However, many datasets for autoimmune diseases with a strong female bias include a female predominance and therefore retain X-linked transcript information, permitting future re-analyses of the data to identify additional differentially expressed X-linked genes in patient samples[56,58]. Future translational investigations in patient cohorts that are clinically well characterized will be instrumental in further defining the spectrum of X-linked genes exhibiting dysregulated expression in a sex-specific and disease-specific manner.

Sex differences in responses to pathogens

Sex differences in the incidence or severity of infectious diseases have been observed for various different pathogens (Supplementary Table 1). Biological sex can impact pathogen replication and transmission due to the differential expression of proteins that the pathogen interacts with or by affecting host immune responses to the pathogen. Although some aspects of sexual dimorphism among infectious diseases may potentially be explained by behavioural differences between male individuals and female individuals (lifestyle choices that affect pathogen exposure, different access to healthcare, among others), hormonal and genetic influences may have a greater impact. The hormonal and genetic contributions responsible for the sex-biased immune responses to various pathogens are summarized in Supplementary Table 1 but have not been well characterized (particularly the genetic component). Rodent models often recapitulate the observed sex bias in humans and have been used to identify sex-specific molecular pathways in response to infection with some pathogens. Male individuals typically exhibit greater incidence for most bacterial, parasitic, some viral and fungal infections (Supplementary Table 1); female individuals have a greater incidence of parasitic worm infections[102–104]. Some pathogens, including HIV, Ebola, *Mycobacterium tuberculosis*, *Cryptosporidium* and SARS-CoV-2, exhibit sex-biased rates of replication,

transmission or co-infections, which probably influence incidence rates[105–110].

The observed male bias in the incidence of infection by most pathogens is typically explained by enhanced inflammatory responses in female individuals. Accumulating evidence suggests that XCI escape of some X-linked immune-related genes in innate and adaptive immune cells contributes towards increased protection of female individuals from bacteria and parasites. Transcription of *ACE2*, one of the receptors targeted by coronaviruses for cellular entry, is affected by both genetic and hormonal factors[109–112], and *ACE2* escapes from XCI in different types of human tissues and in mouse alveolar type 2 cells[72,73]. One study using *Cxcr3* allele-specific reporter mice found that *Cxcr3* escapes XCI in activated T cells, and that following infection with *Leishmania*, biallelic *Cxcr3*-expressing cells produce higher levels of IFNγ, IL-2 and CD69 than monoallelic *Cxcr3*-expressing cells[113]. In general, male individuals have less potent antiviral responses (Table 3), which may be influenced by sex-dependent differences in *UTX* expression levels in NK cells that result in increased numbers of NK cells with reduced functionality in male individuals compared with female individuals[17]. NK cells are necessary for antiviral responses to various viruses including cytomegalovirus, and *Utx* deletion increases lethality following cytomegalovirus infection[17]. It is likely that in addition to *Utx* and *Cxcr3*, other X-linked genes that escape XCI in immune cells impact the response to those pathogens that exhibit sex biases in the incidence of infection (Supplementary Table 1). The identification of these genes may reveal a shared pathway that represents the mechanistic basis for sex-biased immune protection in response to various pathogens.

The contributions of X-linked immune-related genes to sex-biased responses to pathogens are highlighted by sex-specific phenotypes in mice with deletions of particular genes and by patients with specific mutations in such genes who are disproportionally affected by particular infections. For example, the X-linked gene *Ddx3x*, an RNA helicase that impacts RNA processing and transcription and regulates type I IFN production following viral and bacterial infections[114], escapes XCI in female cells. Although male cells contain a Y-linked functional homologue (*Ddx3y*), conditional deletion of *Ddx3x* in mice impairs the ability of male mice (female-specific deletion is embryonic lethal) to respond to *Listeria monocytogenes*[115]. Consistent with these findings, female bone marrow-derived macrophages with conditional homozygous *Ddx3x* deletion are unable to restrict the proliferation of *Listeria*[115]. Deletion of *Ddx3x* in bone marrow-derived macrophages exhibits sex-specific gene expression patterns, and female bone marrow-derived macrophages lacking *Ddx3x* have a greater reduction in cytokine (IL-1, IL-6, IL-12 and TNF) and chemokine expression than male *Ddx3x* mutants[115].

Another example is tuberculosis, which exhibits a male bias with regards to incidence and severity in humans and animal models and is the leading cause globally for bacterial disease (Supplementary Table 1). Mutations in two X-linked genes, *CYBB* and *IKBKG*, increase susceptibility to tuberculosis. Missense mutations in *CYBB*, which encodes the gp91 subunit of the NADPH oxidase complex, result in a hypomorphic protein that impairs respiratory burst activity in macrophages, which is necessary for protection from mycobacteria[116]. IKBKG is the regulatory subunit of the inhibitor of κB kinase (IKK) complex, which regulates canonical NF-κB activation of genes involved in inflammation and immunity. Whereas *IKBKG* mutations result in incontinentia pigmenti, ectodermal dysplasia and various immunodeficiencies, there are some *IKBKG* mutations that predispose male patients to mycobacterial infection through a reduction of IL-12 and IFNγ production[117]. Whether *CYBB*, *IKBKG* or other X-linked genes escape XCI in immune cells during tuberculosis and influence protection in female individuals remains to be determined. Additional research investigating other XCI escape genes, especially those that

Box 3

Mouse models of lupus-like disease that exhibit a sex bias

Spontaneous and induced mouse models of lupus-like disease recapitulate some features of human systemic lupus erythematosus (SLE) (primarily autoantibody production, lymphoid activation and hyperplasia, and lupus nephritis), and some of these models exhibit a sex bias. Male BXSB/*Yaa* mice have a translocation of the telomeric end of the X chromosome containing the *Tlr7* region and 15 neighbouring genes onto the Y chromosome (*Yaa* mutation)[213,214] and acquire a lupus-like phenotype over time. Of note, *Tlr7* knockdown in BXSB/*Yaa* mice abrogates disease development[215], and transgenic overexpression of *Tlr7* on a non-lupus prone background results in lupus-like disease[99]. The BXSB strain also contains mutations in the MHC locus and loci on chromosomes 1, 3 and 13 (*Bsx1–Bsx6*) that contribute to disease activity[216]. The F_1 hybrid New Zealand black–white (NZB/W) mouse model develops spontaneous lupus-like disease that exhibits a female bias (100% females and <40% males develop disease phenotypes by 1 year)[217,218]. NZB/W mice also develop features of Sjögren syndrome and female mice exhibit more extensive salivary gland lesions than male mice[219]. Both X chromosome number and hormones appear to influence disease onset and severity in NZB/W F_1 mice, as oestrogen accelerates disease and testosterone provides anti-inflammatory protection in some studies, but not others[220,221]. In vitro-activated lymphocytes from female NZB/W F_1 mice with lupus-like disease exhibit mislocalized Xist RNA and reduced enrichment of H3K27me3 at the Xi, and sex-biased gene expression differences[56,75]. Backcrosses between NZB/W F_1 and NZW mice generated various recombinant inbred strains of New Zealand mixed (NZM) mice, among which NZM2328 and NZM2410 have earlier disease onset, and NZM2328 displays a stronger female bias for the development of lupus-like disease than NZM2410 (ref. 222). Lupus-like disease can be induced in various mouse strains (with variable efficiency) using intraperitoneal injections of pristane, during which females develop more severe disease phenotypes including autoantibodies targeted at Smith protein, double-stranded DNA, ribosomal P proteins and Argonaute 2 protein, as well as arthritis, immune complex-mediated glomerulonephritis and pulmonary capillaritis[223,224]. Finally, there is a female bias towards autoimmunity in the experimental autoimmune encephalomyelitis model of multiple sclerosis[225].

Table 2 | Immunity-related X-linked genes that are dosage sensitive for autoimmune diseases

Dosage-sensitive X-linked gene	Function	Evidence for XCI escape	Gain-of-function or loss-of-function mouse models and their phenotypes	Aberrant expression in patients with an autoimmune disorder
Adaptive immune system				
CD40LG	Encodes a type II membrane protein that is primarily expressed by $CD4^+$ T cells CD40LG binds to CD40 on B cells, ultimately leading to B cell activation, differentiation and antibody production[137]	Exhibits variable XCI escape in human fibroblasts[138] The *CD40LG* locus is regulated by DNA methylation, and $CD4^+$ T cells treated with DNA methylation inhibitors increase *CD40LG* expression, suggesting reactivation from the Xi (however, direct evidence is lacking)[137,139]	*Cd40lg* overexpression in mice results in chronic inflammation, apoptosis-mediated thymic atrophy, glomerulonephritis, increased level of IgG antibodies and increased lethality[86,140]	Aberrantly overexpressed in $CD4^+$ T cells, $CD8^+$ T cells and B cells with active disease from patients with SLE compared with healthy controls and patients in remission[83,137] $CD4^+$ T cells from a female patient with SLE have increased CD40LG expression compared with $CD4^+$ T cells from male patients[83,137] A patient with *CD40LG* duplication had autoimmune disease (splenomegaly and autoantibodies)[141]
CXCR3	Encodes a chemokine receptor that is expressed by effector T cells and has an important role in T cell trafficking to sites of infection[142] During inflammation, *Cxcr3* is overexpressed by infiltrating $CD4^+$ and $CD8^+$ T cells[143]	Silenced by XCI in human fibroblasts[138] The expression of *CXCR3* is epigenetically regulated by DNA methylation, and $CD4^+$ T cells treated with DNA methylation inhibitors may reactivate expression of *CXCR3* on the Xi (only indirect evidence)[84] Biallelic *Cxcr3* expression in $CD3^+$ T cells from female mice infected with *Leishmania mexicana*[113]	N/A	*CXCR3* is overexpressed in $CD4^+$ T cells in the urine and kidneys from patients with active lupus nephritis[144] $CD4^+$ T cells from female individuals with SLE have increased *CXCR3* mRNA and CXCR3 protein levels[86] Elevated infiltrating $CXCR3^+CD3^+$ T cells in salivary gland tissue of patients with Sjögren syndrome[88]
BTK	Encodes an essential signalling component of the B cell receptor, where BTK activation initiates signalling to facilitate cell proliferation, activation and survival	Silenced by XCI in human fibroblasts[138]	BTK inhibition in NZB/W F_1 mice or in an inducible mouse model of lupus eliminates lupus-like disease symptoms: lower levels of anti-double-stranded DNA antibodies, reduced germinal centre and plasma B cells, reduced complement deposition in kidneys, and reduced inflammatory cytokines[91,145] *Btk* overexpression in mice increases the number of germinal centre B cells and plasma cells, and increases the level of ANAs and immune complexes in kidneys[90,146]	BTK is overexpressed in peripheral blood mononuclear cells from patients with active lupus nephritis[89]
FOXP3	Expression is required for establishment, maintenance, function and identity of T_{reg} cells	Silenced by XCI in human fibroblasts[138]	*Foxp3* overexpression in mice is protective against renal dysfunction[96]	Patients with SLE with increased disease severity exhibit greater numbers of $CD4^+FOXP3^+$ T cells, possibly due to increased T cell activation[94] T_{reg} cells from patients with SLE or SSc express lower levels of *FOXP3* mRNA and FOXP3 protein, which may compromise T_{reg} cell function[92,93] Patients with SSc have reduced numbers of T_{reg} cells[93]
OGT	Encodes a glycosyltransferase that catalyses the addition of *O*-GlcNAc modifications to proteins. It is required for T and B cell activation	–	*Ogt* overexpression in B cells results in enhanced activation, through increased O-GlcNAcylation of BCR-dependent transcription factors[147] *Ogt* deletion in B cells impairs B cell homeostasis, activation and antibody production[148]	Elevated levels of *OGT* mRNA and OGT protein (and hypomethylation of the *OGT* promoter) in patient $CD4^+$ T cells from female patients with SLE compared with those from male patients[84]
Innate immune system				
TASL	Encodes an IFN response gene that is part of the endolysosomal TLR machinery[149,150]	Variable XCI escape in human lymphoblastoid cells[138]	–	Female-biased increased expression in lymphoblastoid cell lines and thyroid tissue[149] TASL is a risk variant for SLE[151] Positive correlation between TASL protein abundance and SLEDAI score in patients with SLE >35 years of age[149]

Table 2 (continued) | Immunity-related X-linked genes that are dosage sensitive for autoimmune diseases

Dosage-sensitive X-linked gene	Function	Evidence for XCI escape	Gain-of-function or loss-of-function mouse models and their phenotypes	Aberrant expression in patients with an autoimmune disorder
Innate immune system (continued)				
TLR7	Encodes an endosomal pattern recognition receptor that binds to guanosine-rich and uridine-rich single-stranded RNA and induces MyD88-dependent activation of type I IFN[152,153]	Variable escape (40–55%) in healthy human B cells, monocytes and plasmacytoid dendritic cells[32] Biallelic expression in B cells from patients with SLE[54] Variable escape in human plasmacytoid dendritic cells; TLR7$^+$ cells with biallelic expression also had elevated expression of IFNβ and IFNα family members[154,155] Variable escape in mouse plasmacytoid dendritic cells; increased biallelic expression in disease NZB/W F_1 mice[59]	Male BXSB/*Yaa* mice have two copies of *Tlr7* and exhibit accelerated lupus-like disease, with ANAs, glomerulonephritis, and splenomegaly[99,100] *Tlr7*-overexpressing mice develop spontaneous autoimmunity, with increased autoantibody production, autoreactive lymphocytes, splenomegaly and lethality[99] *Tlr7* GoF mice (TLR7(Y264H)) have increased responsiveness to guanosine and 2′,3′-cGMP, enhanced TLR7 signalling, increased survival of activated B cells, and cell-intrinsic expansion of age-associated B cells and germinal centre B cells[98]	Biallelic expression of *TLR7* enhances cell responsiveness to TLR7 ligands and leads to an increase in class-switching events (human B cells)[32] Increased *TLR7* expression in female patients with SLE compared with controls[97] Patients with SLE with a TLR7 GoF mutation (Y264H) exhibit an elevated level of ANAs and features of autoimmune disease including refractory autoimmune thrombocytopenia, neuromyelilits optica and inflammatory arthralgias[98]

ANA, antinuclear antibody; BCR, B cell receptor; N/A, not applicable; NZB/W, New Zealand black–white; SLE, systemic lupus erythematosus; SLEDAI, AAA; SSc, systemic sclerosis; TLR, Toll-like receptor; T_{reg} cells, regulatory T cells; XCI, X chromosome inactivation; Xi, inactive X chromosome.

encode chromatin-modifying enzymes, using gain-of-function and loss-of-function mutations in specific types of immune cells will reveal other potential genetic contributors to sex-biased immune responses to pathogens (Supplementary Table 1).

Sex differences in response to vaccination

Compared to male individuals, female individuals often have greater antibody responses, and typically experience more adverse reactions, in response to vaccination[118]. Activation of the innate immune system immediately following vaccination often results in localized inflammation of the injection site. Subsequent activation of the adaptive immune system is also critical for generating an effective memory response to inactivated viruses or viral particles. Female individuals often develop a higher level of inflammation around the vaccine injection site, which may result from sex differences in innate immune activation. However, female individuals also produce antibody profiles that show higher levels of class switching in response to vaccines against influenza, smallpox, measles–mumps–rubella, yellow fever, hepatitis A and hepatitis B, and herpes simplex viruses (Table 3). Female mice injected with inactivated virus produce higher antibody titres and higher numbers of germinal centre B cells and CD8$^+$ and CD4$^+$ T cells in lymph nodes than male mice[119]. Because female individuals often have stronger responses to vaccines against viral and bacterial infections and have more severe reactions, it has been suggested that reduced vaccine doses should be considered for female individuals[33].

Despite ample evidence for sex-biased responses to vaccinations, few X-linked genes that contribute to this finding have been identified. B cells from female mice immunized against influenza virus express higher levels of *Tlr7* (ref. 119), which may result from increased XCI escape and expression from the Xi in response to vaccination. *Tlr7* binds single-stranded RNA from viruses, including SARS-CoV-2, and probably contributes to type I IFN production[120,121]. Loss-of-function *TLR7* mutations in male individuals reduce type I IFN levels and prevent the induction of IFN-stimulated genes, resulting in a high susceptibility to severe disease in response to SARS-CoV-2 infection[120,122–124]. Both mRNA-based and adenoviral vaccines for COVID-19 show a sex bias with regards to adverse events[125]. Here, female individuals are more susceptible to thrombotic thrombocytopenia syndrome in response to vaccination with adenoviral vector-based COVID-19 vaccines[126], whereas young (<30 years of age) human male individuals appear to be more susceptible to myocarditis and pericarditis in response to mRNA vaccines[127]. As myopericarditis appears with similar incidence in male individuals after vaccination with mRNA-based vaccines or with more traditional vaccine formats[127], it might be a general side effect of vaccine-induced inflammation rather than a specific consequence of mRNA vaccination.

Conclusions and perspectives

Biological sex is an important factor for immune responses and immune health, and the importance of understanding hormonal and genetic contributions to sex differences in immunity is increasing. In 2009, about >60% of immunology-related research publications using animal models lacked information about biological sex; in 2014, about 50% of publications reported the biological sex of mouse models[128]. Over half of human immunology publications included male and female samples, yet >90% of these publications lacked sex-specific analyses[129]. Inclusion rates for both sexes in immunological research was 16% in 2009, and increased to 46% by 2019, but suggests that more work is needed to ensure that sex as a biological variable is addressed in future immunological research. Although the effect of sex hormones on sex-specific immune cell function and cytokine production following infection has been investigated for some of the pathogens in Supplementary Table 1, the contribution of the X chromosome to sex-biased immune responses is not well understood. Future experiments examining genes capable of XCI escape in specific immune cell populations, and use of genetic gain-of-function and loss-of-function experiments of immune-related X-linked genes, will reveal important mechanisms of sex differences in infection susceptibility and resulting disease severity. Whereas the sensitivity of X-linked gene dosage for some female-biased autoimmune diseases has been examined, it is still unknown whether X-linked gene dosage influences immune responses to infections with pathogens. Understanding the

Table 3 | Sex-based outcomes to vaccination

Vaccine target	Sex-based outcome	Refs.
Influenza	Females have higher viral titres following seasonal influenza vaccination (using the haemagglutination inhibition assay as a proxy for viral neutralization) In older female individuals, higher haemagglutination inhibition antibody titres are associated with lower hospitalization and mortality, reflecting greater protection than male individuals Female individuals have more local and systemic reactions after seasonal influenza vaccination Female individuals vaccinated with half the dose (seasonal influenza vaccine) generate higher immune responses than male individuals receiving a full vaccine dose Female mice vaccinated with whole-virus trivalent inactivated influenza vaccine produce more IgM and H1N1-specific IgG1 antibodies than male mice	156–161
Smallpox	Female adults (18–40 years of age) who received at least one dose of DRYVAX (lyophilized live vaccine) have higher neutralizing antibody titres Male individuals have had higher peak antibody titres following vaccination with the replication-deficient vaccine IMVAMUNE	162,163
Hepatitis B	Adult female individuals vaccinated with hepatitis B vaccine have higher antibody titres than male individuals	164
MMR	At 14 years post-vaccination with the MMR vaccine, female individuals have higher IgG titres At 2–4 weeks post-vaccination, male individuals have higher antibody responses, but this difference disappears by 10 weeks post-vaccination Female individuals have fewer hospitalization events for measles after the MMR vaccination	165–167
HSV2	Adult female individuals vaccinated with a replication-defective HSV2 vaccine have stronger early inflammatory and type I IFN responses than male individuals Adult female individuals produce more neutralizing antibodies by day 30 post-vaccination with the HSV2 vaccine	168
YFV	Adult female individuals (>18 years of age) have more adverse reactions (local inflammation, fever, pain, headache and fatigue) after YFV vaccine Higher antibody titres in male individuals after YFV vaccine and no difference in adverse events	33,169, 170
Rabies	Adult female individuals have higher antibody responses following rabies vaccine	33
DTP	Female individuals have stronger immune response (<2 years of age) following DTP vaccine, with higher rates of hospitalization and mortality after vaccination	33,171
HPV	Female individuals have higher titres of antibodies following HPV infection Female individuals vaccinated with the quadrivalent HPV vaccine have higher antibody titres against HPV (5–17 years of age)	33,172
SARS-CoV2	Female individuals have stronger immune response and more adverse events following the BNT162b2 mRNA vaccine	125,173

DTP, diphtheria, tetanus, pertussis; HPV, human papillomavirus; HSV2, herpes simplex virus 2; MMR, measles–mumps–rubella; YFV, yellow fever virus.

origins of the sex biases using rodent models that recapitulate the sex differences observed in clinical studies of infectious and autoimmune disease is likely to inform sex-specific treatment strategies.

The interplay between sex chromosomes and sex hormones is likely to influence sex differences in immune responses and sex-biased autoimmune disease. In addition to the Y-linked *Sry* gene, which functions in primary sex determination and the formation of the male gonads responsible for testosterone production, the influence of other X-linked and Y-linked genes on sex hormones in the context of sex-biased immune responses has not been carefully investigated. Given the large number of steroid receptor-binding sites across the genome, including on the X chromosome, it is likely that inflammatory pathways resulting from infections and autoimmunity will promote sex hormone receptor binding to promoters of X-linked immune-related genes, perhaps promoting increased XCI escape in female immune cells. Moreover, androgen and oestrogen receptor expression levels change with cellular activation in immune cells[130], potentially impacting X-linked gene expression and contributing to sex-biased responses. Hormonally induced changes in the expression of X-linked genes are likely to occur on the active X, yet it is possible that XCI escape genes on the Xi could be differentially regulated by androgen and oestrogen receptors in the context of immune activation. Investigation of allele-specific transcriptional changes in response to cellular activation and inflammation is necessary to determine the complex interplay between sex hormones and X-linked gene expression that underlies sex differences in immune responses.

The imbalance of immunomodulatory X-linked gene dosage via perturbations in the XCI machinery in female immune cells provides a plausible and compelling biological mechanism that reconciles the observed sex-biases in microbial pathogen responses and systemic autoimmune disease susceptibility. However, recent data also demonstrate that the XCI machinery itself is a female-specific source of autoantigens that selectively predispose females to the development of systemic autoimmunity[227,226]. XIST RNA, when released from dying cells, has been shown to bind avidly to TLR7 and drive type 1 interferon production by human pDCs, thereby providing a female-specific mechanism of type 1 interferon responses that are often observed across female-biased systemic autoimmune diseases[227]. In addition, the introduction of a modified Xist transgene without X silencing capabilities into autoimmune-prone male mice was able to modify their susceptibility to pristane-induced lupus-like disease, in association with a splenic expansion of an inflammatory B cell subset implicated in systemic autoimmunity[227,228] Furthermore, individuals with systemic autoimmune diseases including SLE, SSc and dermatomyositis were found to have autoantibodies against some of XIST binding proteins, although male patients also had evidence of these autoantigens[227]. Collectively, these data indicate that the very expression of XIST RNA[228] and its binding partners[227] in female individuals may provide a relevant source of autoantigens that enhance the potency of immune responses in females.

In conclusion, there is clear evidence demonstrating that the relative abundance of sex hormones, the dosage of immunomodulatory X-linked genes, and the expression of the XCI ribonucleoprotein machinery can all influence sex-biased immune responses. Understanding how these diverse mechanisms cooperate and synergize with one another will undoubtedly enhance our understanding of the mechanisms that contribute to sex-biased immune functions and will ultimately facilitate new therapeutic approaches for effective precision medicine.

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Author contributions

All authors researched data for the article and contributed substantially to discussion of the content. M.C.A. wrote the article. All authors reviewed and edited the manuscript before submission.

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