Sex differences in immune responses

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Abstract | Males and females differ in their immunological responses to foreign and self-antigens and show distinctions in innate and adaptive immune responses. Certain immunological sex differences are present throughout life, whereas others are only apparent after puberty and before reproductive senescence, suggesting that both genes and hormones are involved. Furthermore, early environmental exposures influence the microbiome and have sex-dependent effects on immune function. Importantly, these sex-based immunological differences contribute to variations in the incidence of autoimmune diseases and malignancies, susceptibility to infectious diseases and responses to vaccines in males and females. Here, we discuss these differences and emphasize that sex is a biological variable that should be considered in immunological studies.

Sex is a biological variable that affects immune responses to both self and foreign antigens (for example, those from fungi, viruses, bacteria, parasites and allergens). The sex of an individual is defined by the differential organization of chromosomes, reproductive organs, and sex steroid levels; it is distinct from gender, which includes behaviours and activities that are determined by society or culture in humans. Male and female differences in immunological responses may be influenced by both sex and gender, with sex contributing to physiological and anatomical differences that influence exposure, recognition, clearance, and even transmission of microorganisms. By contrast, gender may reflect behaviours that influence exposure to microorganisms, access to healthcare or health-seeking behaviours that affect the course of infection. Although we acknowledge that both sex and gender influence the immune response, the focus of this Review will be on the biological factors that influence immunological differences between the sexes. Despite a growing body of literature illustrating sex-based differences in immune responses, immunology ranks the lowest of ten biological disciplines for reporting the sex of animal or human subjects in published papers, with fewer than 10% of articles analysing data by sex[1]. The field of sex-based biology is undergoing a revolution, in which research funding agencies and journals have launched policies to promote greater consideration, reporting and analyses of sex and gender in the biomedical sciences in an effort to improve rigour and reproducibility (BOX 1).

It is increasingly important to acknowledge sex differences in immune responses when we consider the marked differences seen between males in females in various diseases. For instance, 80% of autoimmune disease occurs in females, women with acute HIV infection have 40% less viral RNA in their blood than men, men show an almost twofold higher risk of death from malignant cancer than women and antibody responses to seasonal influenza vaccines are consistently at least twice as strong in women than men. Generally, adult females mount stronger innate and adaptive immune responses than males. This results in faster clearance of pathogens and greater vaccine efficacy in females than in males but also contributes to their increased susceptibility to inflammatory and autoimmune diseases. In this Review, we explain how these immunological differences between the sexes reflect hormonal, genetic and environmental effects on the immune system that can change throughout life in humans.

Phylogeny of sex differences in immunity

Mounting immune responses that are necessary for the recognition, response and clearance of pathogens requires metabolic resources that might otherwise be used for other biological processes, such as growth, maintenance of secondary sex characteristics and reproduction. Trade-offs are likely to exist for life strategies that affect survival and reproduction[2]. Several theories posit that increased pathogen loads and reduced immune function among males are an adverse side effect of positive selection for other traits or characteristics that increase reproductive success and survival[2]. Sex differences in immune responses have evolved in diverse species ranging from insects to lizards, birds and mammals; in all of these species, both innate and adaptive immune responses are typically lower in males than in females (TABLE 1). In *Drosophila melanogaster*, for example, many of the genes that encode for innate signalling proteins are found on the X chromosome and show sex-specific induction following fungal or bacterial infection[3,4]. In lizards, the

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Box 1 | **A brief history of sex and gender-based research in the US**

The history of excluding females from clinical studies is reflected in the 1977 US Food and Drug Administration (FDA) guidelines advising that women of childbearing potential should be excluded from drug trials. These recommendations resulted in inadequate representation of women in clinical trials for decades. In the early 1990s, the FDA and the National Institutes of Health (NIH) in the US, with advocacy from US Congresswomen, recommended that clinical trials should include female subjects. Although women are now included in clinical trials of drugs, devices and biologics, there remains inadequate analysis of whether outcomes differ between men and women or boys and girls. Of drugs withdrawn from the US market from 1997–2000, the US Government Accountability Office (GAO) reported that 8 out of 10 drugs taken off the market had greater adverse effects in women. In 2015, the US GAO documented that although more women than men currently enrol in NIH-funded clinical research, the NIH does not ensure that these studies are designed to identify differences between men and women in disease processes and responses to treatment. Preclinical studies in animal models and cell culture systems could help to prevent these costly mistakes but, here too, analysis of potential sex effects has been lacking. Following behind policy changes in Canada and Europe, in 2015 the NIH announced new policies to ensure that sex is considered as a biological variable in preclinical research in an effort to increase rigour and reproducibility.

phagocytic activity of macrophages is greater in females than in males owing to the suppressive effects of androgens on male macrophage activity[5]. In birds, females exhibit higher antibody and cell-mediated immune responses to immune challenges, and these effects are often most pronounced during the mating season when male testosterone concentrations are highest[6,7].

Sex differences in innate immunity in mammals

Among mammals, males and females differ in their innate immune responses, which suggests that some sex differences may be germline encoded (TABLE 2). For example, innate detection of nucleic acids by pattern recognition receptors (PRRs) differs between the sexes. The Toll-like receptor 7 (*TLR7*) gene, encoded on the X chromosome, may escape X inactivation resulting in higher expression levels of *TLR7* in females than males[8]. Exposure of peripheral blood mononuclear cells (PBMCs) to TLR7 ligands *in vitro* causes higher production of interferon-α (IFNα) in cells from women than from men[9]; in addition, plasmacytoid dendritic cells (pDCs) from female humans and mice have higher basal levels of IFN regulatory factor 5 (IRF5) and IFNα production following TLR7 ligand stimulation[10]. Transcriptional regulation of IRF5 in female mice is under the control of signalling through oestrogen receptor-α (ERα)[10]. Stimulation of DCs with CpG, a TLR9 ligand, results in no sex bias in IFNα production[9]. Transcriptional analyses reveal sex differences in the expression of genes along TLR pathways and induction of type I IFN responses. Following either vaccination in adult humans or virus challenge in adult rats, the expression of TLR-pathway and pro-inflammatory genes (for example, *TLR7*, myeloid differentiation primary response gene 88 (*MYD88*), retinoic acid inducible gene-I (*RIGI*), *IRF7*, *IFNB*, Janus kinase 2 (*JAK2*), signal transducer and activator of transcription (*STAT3*), nuclear factor-κB (*NFKB*), *IFNG* and tumour necrosis factor (*TNF*)) is higher in female than male PBMCs from humans and tissues from rats[11,12]. Putative androgen response elements (AREs) and oestrogen response elements (EREs) are present in the promoters of several innate immunity genes, suggesting that sex steroids may directly cause dimorphic innate immune responses[12].

The production of cytokines and chemokines by innate immune cells also differs between the sexes. Activation of TLR9 with viral or synthetic ligands in PBMCs from human males results in greater interleukin-10 (IL-10) production compared with PBMCs from females, which is positively correlated with androgen concentration in males[13]. PBMCs from human males produce more TNF than PBMCs from females following lipopolysaccharide (LPS) stimulation[14,15]. Neutrophils from human males express higher levels of TLR4 and produce more TNF than female neutrophils both constitutively and following activation with LPS[16]. Peritoneal macrophages from male mice express higher levels of TLR4 and produce more CXC-chemokine ligand 10 (CXCL10) following LPS stimulation than macrophages from females[17]. Peritoneal macrophages isolated from female rodents produce higher levels of anti-inflammatory prostanoids than male-derived cells following LPS treatment[17]. Because TLR4 expression is greater on immune cells from males than females, stimulation with LPS results in greater pro-inflammatory cytokine production by male immune cells, which can be reversed by removal of androgens in male rodents[18]. By contrast, higher expression of TLR7 in immune cells from females compared with males seems to cause greater cytokine production by female immune cells and is regulated by sex chromosome expression. The sex differential expression of PRRs is crucial for interpreting sex-specific activity of innate immune cells following stimulation.

The number and activity of cells associated with innate immunity differ between the sexes. Males have higher natural killer (NK) cell frequencies than females[19]. The phagocytic activity of neutrophils and macrophages is higher in females than males[20]. Antigen-presenting cells (APCs) from females are more efficient at presenting peptides than APCs from males[21]. Finally, sex differences are also seen in innate lymphoid cells (ILCs), which are innate-like lymphocytes that regulate an array of tissue immune responses through the production of effector cytokines. Dysregulation of ILCs is linked to the development of autoimmune diseases, and females reportedly have reduced numbers of type 2 ILCs, which is hypothesized to contribute to their increased susceptibility to demyelination in a mouse model of multiple sclerosis[22].

Sex differences in adaptive immunity in mammals

Sex influences multiple aspects of adaptive immunity (FIG. 1; TABLE 2). The thymus plays a pivotal part in the development of the adaptive immune system by producing the peripheral T cell pool. Early in life, male rats have larger thymuses, greater thymocyte counts and differential distribution of thymocyte subsets compared with female rats[23,24].

Among adult humans, sex differences in lymphocyte subsets — including B cells, $CD4^+$ T cells and $CD8^+$ T cells — are described for multiple ethnic groups

Table 1 | **Sex differences in immune responses in different species**

Common name	Species	Immune component	Sex difference
Sea urchin	*Paracentrotus lividus*	Number of immunocytes, cytotoxic activity, phagocytosis and haemolysis	Greater in females than in males
Fruit fly	*Drosophila melanogaster*	Activation of Toll and immune deficiency signalling	Greater in females than in males
Scorpionfly	*Panorpa vulgaris*	Haemolysis and phagocytosis	Greater in females than in males
Wall lizard	*Podarcis muralis*	Macrophage phagocytosis	Greater in females than in males
Eurasian kestrels	*Falco tinnunculus*	Hypersensitivity responses	Greater in females than in males
Great tit	*Parus major*	Hypersensitivity responses	Greater in females than in males
House mouse	*Mus musculus*	Pro-inflammatory cytokine responses, T cell proliferation and antibody responses	Greater in females than in males
Rhesus macaque	*Macaca mulatta*	Pro-inflammatory cytokine responses and antibody responses	Greater in females than in males
Human	*Homo sapiens*	Type I interferon activity, T cell numbers and antibody responses	Greater in females than in males

including Europeans, Asians, and Africans. Females (both children and adults) have higher $CD4^+$ T cell counts and higher CD4/CD8 ratios than age-matched males[19,25–27]; whereas males have higher $CD8^+$ T cell frequencies[25–27]. Following *in vitro* stimulation of PBMCs, women have higher numbers of activated $CD4^+$ T cells and $CD8^+$ T cells and proliferating T cells in peripheral blood compared to men[19,28]. Transcriptional analyses indicate greater cytotoxic T cell activity in adult females, with PMA–ionomycin-stimulated T cells from women upregulating more antiviral genes (such as *IFNG*, *RIGI*, *SPINK5*, *OAS1* and *IFI6*) and pro-inflammatory genes (for example, *IL12RB2*, *IL1F5*, *CXC3CL1*, *CXCL2* and *IL16*) compared with T cells isolated from men[29]. Notably, half of the activated genes in female T cells have EREs in their promoters[29].

The activity and distribution of $CD4^+$ T cell subsets differ between the sexes. Adult female mice produce higher levels of T helper 1 (T_H1)-type cytokines (for example, IFNγ) than males, at least following parasitic infections, such as *Leishmania major* and *Plasmodium chabaudi*, in which females are better protected[30]. Polyclonal activation of human PBMCs with the mitogen phytohaemagglutinin (PHA) results in higher production of T_H2-type cytokines, including IL-4 and IL-10 in female PBMCs than in male PBMCs[31]. The T_H1–T_H2 dichotomy in males and females may not always hold true in humans. Naive $CD4^+$ T cells from human females preferentially produce IFNγ upon stimulation, whereas naive T cells from males produce more IL-17 (REF. 32). Expression of IL-17A is higher in males[29] or females[28], depending on the stimulation and purity of the T cell population. Mouse studies investigating sex differences in regulatory T (T_{reg}) cells describe contradictory results regarding organ-specific T_{reg} cell frequencies in various diseases, whereas human studies suggest there are higher numbers of T_{reg} cells in healthy adult males compared with females[33].

Regardless of age, females tend to show greater antibody responses than males, higher basal immunoglobulin levels and higher B cell numbers[19,34,35]. Global analysis of B cell gene-expression signatures reveal that the majority of genes differentially expressed between the sexes are significantly upregulated in B cells from females compared with males[36].

Genetic mediators

Sex chromosomes. Many genes on the X chromosome regulate immune function and play an important role in modulating sex differences in the development of immune-related diseases[37] (BOX 2). These genes code for proteins ranging from PRRs (for example, *TLR7* and *TLR8*) to cytokine receptors (for example, *IL2RG* and *IL13RA2*) and transcriptional factors (for example, *FOXP3*). The Y chromosome also contains numerous regulatory response genes, and Y chromosome polymorphisms affect sex-dependent susceptibility to viral infection[38].

The *SRY* gene on the Y chromosome causes testes formation and testosterone synthesis, leading to male phenotypic development, whereas the absence of *SRY* expression results in ovaries and female-typic development. The 'four core genotypes' (FCG) mouse model was developed to investigate the impact of sex chromosomes (XX versus XY) and gonadal type (testes versus ovaries) on phenotypes. Deletion of *Sry* from the Y chromosome results in XY minus (XY^-) FCG mice that are gonadal females (that is, with ovaries). Insertion of the *Sry* transgene onto an autosome in XX or XY^- FCG mice (XX*Sry* and XY^-*Sry*) results in gonadal males (that is, with testes). Depletion of gonadal steroids by gonadectomy of FCG mice unmasks effects of sex chromosome complement on multiple functions, including susceptibility to autoimmune disease and viral infection[39,40]. In experimental autoimmune encephalitis (EAE) and lupus, for example, the presence of the XX sex chromosome complement worsens disease progression, relative to that in the XY mice, and results in decreased production of IL-4, IL-5 and IL-13, but increased IL-13Rα2 expression on DCs[39].

Klinefelter and Turner syndromes are two inherited disorders that further exemplify the effects of the X chromosome on immunity. Klinefelter syndrome occurs when males have an extra X chromosome, resulting in low testosterone, increased gonadotrophins and elevated oestrogen concentrations. Immunologically, men with Klinefelter syndrome respond more like females, with higher immunoglobulin concentrations, $CD4^+$ T cell numbers, CD4/CD8 T cell ratios and B cell numbers than XY male controls[41]. The immunological effects of Klinefelter syndrome are reversed by testosterone therapy[41], illustrating that both sex chromosomes and sex steroids regulate immune responses. By contrast, women with Turner syndrome (that is, who have only one X chromosome (X0) or have major X chromosome deletions[42]) have lower IgG and IgM levels and

Table 2 | **Sex differences in innate and adaptive immune responses in adults***

Immune component	Characteristic	Sex difference
Sex differences in the innate immune system		
TLR pathways	TLR pathway gene expression	Higher in females
	TLR7 expression	Higher in females
	IL-10 production by TLR9-stimulated PBMCs	Higher in males
APCs	APC efficiency	Higher in females
Dendritic cells	TLR7 activity	Higher in females
	Type 1 interferon activity	Higher in females
Macrophages	TLR4 expression	Higher in males
	Activation	Higher in females
	Phagocytic capacity	Higher in females
	Pro-inflammatory cytokine production	Higher in males
	IL-10 production	Higher in females
Neutrophils	Phagocytic capacity	Higher in females
	TLR expression	Higher in males
NK cells	NK cell numbers	Higher in males
Sex differences in the adaptive immune system		
Thymus	Size of thymus	Larger in males
T cells	$CD4^+$ T cell counts	Higher in females
	CD4/CD8 T cell ratio	Higher in females
	$CD8^+$ T cell counts	Higher in males
	Number of activated T cells	Higher in females
	T cell proliferation	Greater in females
	Cytotoxic T cells	Increased cytotoxic activity in females
	T_H1 versus T_H2 cell bias	T_H2 cell bias in females, T_H1 cell bias in males
	T_{reg} cell numbers	Increased in males
B cells	B cell numbers	Increased in females
Immunoglobulins	Antibody production	Higher in females

APC, antigen-presenting cell; IL, interleukin; NK, natural killer; PBMCs, peripheral blood mononuclear cells; T_H, T helper; TLR, Toll-like receptor; T_{reg}, regulatory T. *Based on data from humans and rodents and primary cell cultures.

lower T cell and B cell levels compared to XX females[43]. Both patients with Klinefelter syndrome and patients with Turner syndrome show increased development of autoimmune disease, pointing to a major role for the X chromosome in influencing susceptibility to autoimmunity[42].

MicroRNAs and long non-coding RNA. Much of the mammalian genome encodes for transcripts that are not translated into proteins, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). Despite the fact that male and female invertebrates and higher organisms differentially express miRNAs, few studies have addressed the role of miRNAs in sex differences in diseases[44]. The X chromosome contains 10% of the ~800 miRNAs in the human genome, whereas the Y chromosome only contains 2 miRNAs[45]. MiRNAs — including miRNA-18 and miRNA-19, which are encoded on the X chromosome — play a role in sex differences in immune responses[46]. MicroRNA expression can be under sex hormone control[47]. The high density of miRNAs on the X chromosome means that females may express more owing to incomplete X inactivation (BOX 2), further contributing to sex differences in susceptibility to certain diseases.

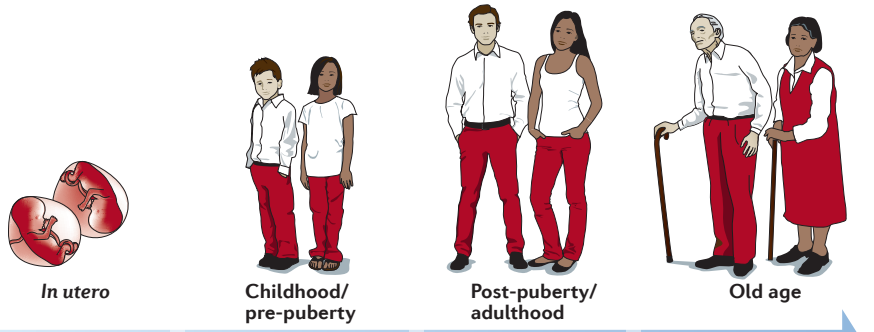
The lncRNAs play a vital role in the regulation of multiple immunological processes, including transcriptional regulation of innate and adaptive immunity[48] and as a catalyst of X inactivation in a manner that has been shown to be sex-differential[49].

Genetic polymorphisms. Polymorphisms or variability in sex chromosome and autosomal genes encoding immunological proteins can have sex-differential effects on immunity. For example, sex-based differences in HLA alleles and genes that encode for IL-4, IL-10 and the IL-12 receptor, have each been associated with differential antibody responses to vaccines against measles, mumps, hepatitis A, tetanus and diphtheria in children and adults[11]. Whether sex-based differences in the expression of gene variants are caused by differential selection pressures acting on each sex, hormone-dependent effects, or epigenetic mechanisms remain to be determined but, because these differences are apparent early in life and remain over the life course, genetic as opposed to hormonal mechanisms are likely to be involved[50].

Hormonal mediators

Oestradiol. Levels of oestrogen, for example, 17β-oestradiol (E2), are variable during the menstrual cycle, high during pregnancy and low after menopause in females. ERs are expressed in various lymphoid tissue cells, in lymphocytes, macrophages, and DCs. The two ER subtypes for classical oestrogen signalling, ERα and ERβ, exhibit differential expression among immune cell subsets; ERα is highly expressed in T cells and ERβ is upregulated in B cells[51]. Non-classical ER signalling also occurs in immune cells, enabling protein–protein interactions between ERs and ERE-independent transcription factors, including NF-κB, specific protein 1 (SP1) and activator protein 1 (AP-1)[52]. Differential effects of oestrogens on immune function reflect not only oestrogen concentration but also the density, distribution and type of ERs in immune cells.

E2 affects many aspects of innate immunity (TABLE 3), including the functional activity of innate immune cells that influence downstream adaptive immune responses. Treatment of either humans or mice with E2 increases neutrophil numbers in the blood and lungs, respectively[53,54]. Exposure of NK cells to E2 *in vitro* enhances production of IFNγ and overall cytotoxicity[55], but also downregulates expression of NK cell surface activation markers and FAS ligand (FASL), and reduces their secretion of granzyme B in mice[56]. E2 has bipotential effects on monocytes and macrophages derived from humans, with low doses enhancing the production of pro-inflammatory cytokines (such as IL-1, IL-6 and TNF) and high concentrations reducing their production of



Innate immunity	• Increased inflammatory responses in males	↑ Inflammation in males ↑ NK cells in males	↑ Inflammation in females ↑ NK cells in males	↑ Inflammation in males ↑ IL-10 in females ↑ NK cells in females
Adaptive immunity	• Increased IgE levels in males	• CD4/CD8 ratios and $CD4^+$ T cell numbers equal • $CD8^+$ T cell numbers equal • IgA levels in males ≥ females • IgM levels in males ≥ females • IgG and IgM levels equal • B cell numbers equal • T_{reg} cell numbers in males ≥ females	• CD4/CD8 ratios and $CD4^+$ T cells ↑ in females • $CD8^+$ T cells ↑ in males • T cell activation/ proliferation ↑ in females • T_{reg} cells ↑ in males • B cells ↑ in females • Immunoglobulins ↑ in females	• CD4/CD8 ratios and $CD4^+$ T cells ↑ in females • $CD8^+$ T cells ↑ in males • T cell activation/ proliferation ↑ in females • T_{reg} cells ↑ in males • B cells ↑ in females • Immunoglobulins ↑ in females

Figure 1 | **Changes in immune responses in human males and females over the life course.** Multiple immunological factors vary between the sexes throughout the course of life. For certain factors (for example, pro-inflammatory responses), the sex differences change at puberty and then wane in later life suggesting hormonal effects. For other factors the sex difference remains constant from birth to old age (for example, higher numbers of $CD4^+$ T cells and CD4/CD8 T cell ratios in females). The paucity of studies in this area is notable, particularly *in utero* sex differences in which results are conflicting. IL-10, interleukin-10; NK, natural killer; T_{reg}, regulatory T.

these cytokines[57]. E2 also enhances the expression of PRRs, including TLR4, on the surface of peritoneal macrophages in rodents[58]. *In vitro* E2 exposure facilitates the differentiation of bone marrow precursor cells into functional $CD11c^+$ DCs[59] and increases the synthesis of CXCL8 and CC-chemokine ligand 2 (CCL2) by immature DCs in mice[60]. Treatment of ovariectomized mice with physiological doses of E2 increases production of pro-inflammatory cytokines by $CD11c^+$ DCs[61,62]. E2 acts primarily through ERα, not ERβ, to regulate DC differentiation in mice[59]. In response to granulocyte–macrophage colony-stimulating factor (GM-CSF) in human monocyte cell systems, E2 promotes differentiation of monocytes into inflammatory DCs, which show increased production of IFNα and pro-inflammatory cytokines, increased TLR7 and TLR9 signalling, and greater internalization and presentation of antigen to naive T cells[63]. This is most likely to contribute to the greater type I IFN activity seen in immune cells from females than in males.

E2 enhances both cell-mediated and humoral immune responses (TABLE 3). Generally, low E2 concentrations promote T_H1-type responses and cell-mediated immunity, whereas high E2 concentrations augment T_H2-type responses and humoral immunity in diverse species and cell culture systems[64]. Binding of E2 to ERs increases *Ifng* transcription via EREs in the promoter region of the *Ifng* gene[65]. Low dose E2 also upregulates mitogen activated protein kinase (MAPK), T-bet, and select miRNAs to increase production of IFNγ by T cells, an effect reversed by the ER antagonist ICI 182,780 in murine studies[66–68]. E2 regulates pro-inflammatory responses that are transcriptionally mediated by NF-κB through a negative feedback and/or transrepressive interaction with NF-κB[64].

Exogenous E2 enhances the expansion of T_{reg} cell populations in mice and healthy women and, *in vitro*, E2 increases the number of T_{reg} cells generated from PBMCs[69,70]. Treatment of mice with high doses of E2 decreases IL-17 production by T_H17 cells[71], whereas ovariectomy of female mice increases T_H17 cell numbers and IL-17 production[72]. E2 at physiological concentrations also stimulates humoral responses to infection[73]. Numbers of antibody-secreting cells and antibody levels are highest before ovulation in females[73]. Oestrogen also induces somatic hypermutation and class switch recombination in B cells via the upregulation of activation-induced deaminase[74].

Progesterone. Progesterone (P4) is produced by the corpus luteum during the menstrual cycle and at high levels by the placenta during pregnancy. P4 signals through

Box 2 | **Sex chromosomes and X inactivation**

In humans, sex chromosomes are heterologous in males (XY) and homologous in females (XX). The human Y chromosome contains approximately 100 genes, including *SRY*, which encodes for the testis determining factor, and regulatory genes that may be important for immune responses in autoimmune and infectious diseases[38,144]. The human X chromosome contains over 1,100 annotated genes, representing approximately 5% of the human genome, and includes a significant number of immune related genes, such as interleukin 2 (IL-2) receptor-γ chain, IL-3 receptor-α chain, IL-13 receptor-α chains, Toll-like receptor 7 (TLR7), TLR8, GATA1, IL-1 receptor-associated kinase 1 (IRAK1), CD40 ligand and FOXP3 (REF. 145). Several crucial transcriptional and translational control effectors, that function downstream of activated cytokine receptors, are encoded on the X chromosome. The implications are that X-linked genes are determinants of sex differential immune responses. For genes on the X chromosome, outside of the pseudoautosomal regions, one copy has to be silenced to ensure only a single copy functions in each sex. Inactivation is initiated by the X-inactive specific transcript (*XIST*) gene. Approximately 15% of X genes in humans and 3% in mice escape X inactivation and are found in higher copy number in females than males. For X-linked genes that are inactivated in females, the random process of inactivation of copies derived from the maternal or paternal X chromosome results in a mosaic in females, but not in males. Genomic imprinting is an epigenetic mechanism that is responsible for an imbalance in expression of maternal and paternal inherited genes according to the parent-of-origin. It varies in different tissues and at different developmental stages. In mice, the expression levels of certain imprinted genes vary between the sexes[146]. In addition to sex differences in transcription, there are also sex differences in post-transcriptional mechanisms.

the progesterone receptor and to a lesser extent, through glucocorticoid and mineralocorticoid receptors. progesterone receptors are present on many different immune cell types, including NK cells, macrophages, DCs, and T cells[75].

P4 has broad anti-inflammatory effects (TABLE 3). P4-exposed macrophages and DCs have a lower state of activation and produce lower amounts of IL-1β and TNF compared with untreated cells [76,77]. P4 treatment of mouse bone marrow-derived macrophages induces the expression of FIZZ1 and YM1, both markers of alternatively activated macrophages, and reduces production of inducible nitric oxide synthase (iNOS) and nitric oxide (NO)[78]. TLR and the NF-κB pathways can also be antagonized by the action of P4 (REFS 76,79). Treatment of human NK cells with P4 reduces activation and production of IFNγ via caspase-dependent apoptosis[80]. Progesterone can promote skewing of $CD4^+$ T cell responses from T_H1-type towards T_H2-type responses, as characterized by increased IL-4, IL-5, and IL-10 production[81]. When cord blood cells are treated with P4, the percentage of $FOXP3^+$ T_{reg} cells increases, while T_H17 cell frequencies decline[82]. Whether P4 contributes directly to male–female differences in the skewing of $CD4^+$ T cell responses requires consideration.

Androgens. Androgens, including dihydrotestosterone (DHT) and testosterone, occur in higher concentrations in post-pubertal men than women, and they generally suppress immune cell activity[30] (TABLE 3). Exposure to testosterone *in vivo* reduces NK cell activity in mice[83]. Surface expression of TLR4 on macrophages is reduced by exposure to testosterone both *in vitro* and *in vivo*, driving increased susceptibility to endotoxic shock following gonadectomy of male mice[18]. Testosterone reduces the synthesis of TNF, iNOS and NO by macrophages[84]. Testosterone and DHT increase IL-10 and transforming growth factor-β (TGFβ) synthesis, causing increased anti-inflammatory responses via androgen receptor signalling[84,85]. Androgens also suppress pro-inflammatory responses by reducing extracellular signal-regulated kinases and leukotriene formation in neutrophils[86].

Men with androgen deficiencies have higher concentrations of inflammatory cytokines (for example, IL-1β, IL-2 and TNF), antibody titers and CD4/CD8 T cell ratios than men with normal testosterone levels[87–90]. Men treated with a gonadotropin-releasing hormone antagonist, which significantly reduces testosterone levels, have lower peripheral blood T_{reg} cell counts and higher NK cell counts compared with placebo-treated men or men treated with both the gonadotropin-releasing hormone antagonist and exogenous testosterone[91]. Castrated male mice have higher numbers of $CD4^+$ and $CD8^+$ T cells[92] and higher numbers of macrophages and antigen-specific $CD8^+$ T cells following viral infection than gonadally intact males[93]. Treatment of female mice with testosterone inhibits secretion of IFNγ by natural killer T cells[94].

The immunosuppressive effects of androgens may reflect the inhibitory effects of androgen receptor signalling on transcriptional factors for pro-inflammatory and antiviral cytokines[95]. Androgens also enhance the expression of peroxisome proliferator-activated receptor-α (PPARα) in T cells by engaging AREs in the promoter of the PPARα gene, which repress the activity of NF-κB and JUN to control inflammation[96]. Taken together, these studies illustrate that sex steroids are potent regulators of immune responses.

Environmental mediators

Although genes and hormones are the most well characterized mediators of sex differences in immune responses, environmental factors can also modulate the functioning of the immune system differentially between males and females.

Nutrition. The nutritional environment of the fetus can have differential effects depending on its sex. Maternal micronutrient supplementation during pregnancy in a Gambian placebo-controlled study reported sex differences in CpG methylation of genes involved in immunity and defence against infection (for example, genes encoding CD4, defensins and genes associated with IFN signalling), and female fetuses were most affected in the supplemented group whereas males were most affected in the non-supplemented group[97]. The study demonstrates that sex-differential developmental trajectories commence *in utero* and persist to 9 months of age, indicating long-term epigenetic reprogramming in relation to nutrition during pregnancy. A high-fat diet also enhances, whereas prenatal exposure to famine reduces, placental gene expression and DNA hypomethylation to a greater extent in female than in male fetuses[98]. Several studies also suggest that the immunomodulatory effects of breast milk may benefit infant females more than males, with breastfeeding reducing the risk of neonatal respiratory tract infection in female but not male infants[99].

Table 3 | **Effects of sex steroid hormones on innate and adaptive immunity**

Immune component	Effect of sex hormones*		
	Oestradiol	Progesterone	Androgens
TLRs	↑TLR4, TLR7 and TLR9	↓TLR3 and TLR7	↓TLR4
Macrophages	↑TLR4	↓iNOS and NO ↑FIZZ1 and YM1	↓iNOS/ NO ↓TNF
NF-κB	↓Activity	↓Activity	↓Activity
DCs	↑Activation ↑TLR7and TLR9 ↑CCL2 ↓CXCL10 ↓IFNα	↓CD40, CD80, CD86 and ↑CD11c ↑IL-18 and IL-10	ND
Neutrophils	↑Numbers ↑Degranulation ↑Elastase release	ND	↑Numbers ↓Kinases and leukotriene formation
NK cells	↑IFNγ ↑Granzyme B ↓FASL	↑Numbers ↑Apoptosis (caspase dependent)	ND
Eosinophils	↓Numbers ↓Mobilization	↑Numbers	ND
Inflammatory cytokines	Low oestrogen: ↑IL-1β, IL-6, andTNF High oestrogen: ↓L-1β, IL-6 and TNF	↓TNF and IFNγ ↑IL-6	↑IL-1β and IL-2 ↓TNF
Suppressive cytokines	↑IL-4, IL-10 and TGFβ	↑IL-4, IL-5 and TGFβ	↑IL-10 and TGFβ
Chemokines	↓CCL2 ↑CXCL1	↓CXCL2	↓CCL3
T_H1 cells	Low oestradiol: ↑Activity	↓Activity	↓IFNγ
T_H2 cells	High oestradiol: ↑Activity	↑Activity	↓IL-4 and IL-5 ↓GATA3
T_H17 cells	↓Numbers ↓IL-17	↓Percentages	↑IL-17
T_{reg} cells	↑Numbers	↑Percentages	↑Numbers
$CD8^+$ T cells	↑Response	↓Response	↓Numbers ↓Activity
B cells	↑IgG and IgM	↓CD80 and CD86	ND
Antibody responses	↑Response	↑Total antibody ↓Autoantibodies	↓Response

CCL, CC-chemokine ligand; CXCL, CXC-chemokine ligand; DCs, dendritic cells; FASL, FAS ligand; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; ND, not defined; NF-κB, nuclear factor-κB; NK, natural killer; NO, nitric oxide; TGFβ, transforming growth factor-β; T_H, helper; TLR, Toll-like receptor; TNF, tumour necrosis factor; T_{reg}, regulatory T. *There is growing evidence that immune cells have sex hormone receptors and can respond directly to the presence, absence or changes in the concentrations of sex steroid hormones. Androgens (including testosterone), oestrogens (including 17β-oestradiol), and progesterone can have distinct and overlapping effects on the recruitment and activity of diverse immune cell populations in humans, rodents and primary cell culture systems. Generally, testosterone and progesterone are anti-inflammatory, suppressing several of the immune responses necessary for inflammation. Oestradiol has bipotential effects: low concentrations of oestradiol (for example, during the follicular stage of the reproductive cycle) can be pro-inflammatory, whereas high concentrations of oestradiol (for example, during the luteal phase of the reproductive cycle or during pregnancy) can be anti-inflammatory.

There is accumulating evidence that micronutrients act differently in males and females. Perinatal and postnatal vitamin B, vitamin C and vitamin E supplements are associated with a 32% reduction in mortality among females but not males in a randomized placebo-controlled trial of Tanzanian mothers infected with HIV[100]. Studies conducted in African and Asian infants suggest that females may benefit more than males from maternal micronutrient supplements[101,102]. Vitamin A supplementation (VAS), given with measles vaccination to children between 6 and 23 months of age, have sex differential immunomodulatory effects compared to a placebo, including decreased leukocyte subsets in males, and increased numbers of leukocytes and IFNγ production by *ex vivo* stimulated cells from females[103].

Microbiota. A perturbed microbiome — referred to as dysbiosis — contributes to various disease processes including inflammation and diabetes. Sex influences the host microbiome outside of the reproductive tract, which probably involves sex steroid hormones[104,105]. During early life, sex does not influence the microbiome composition. Deep sequencing of colonic contents in pre-pubescent mice report no sex difference in bacterial community composition, suggesting that sex does not influence the microbiome in this age group[106]. A number of mouse studies, however, show sex differences in host gene expression in the gastrointestinal tract before puberty, demonstrating that sex-specific gene regulation occurs even in the absence of high levels of circulating sex hormones[106]. After puberty, female rodents have lower frequencies of *Bacteroidetes* than males[104,105].

In a mouse model of spontaneous type 1 diabetes, adoptive transfer of gut commensals from male mice into females resulted in systemic hormonal changes and protected against disease[104,105]. Similar to what is seen in mice, the human female microbiome is less abundant in *Bacteroidetes* spp. than males[107]. A study specifically analysing for a sex–diet interaction in diverse vertebrate species including fish, mice, and humans confirmed that diet has sex-specific effects on the gut microbiome in two species of fish, affects *Fusobacteria* spp. levels in humans, but does not seem to affect the microbiome in laboratory mice[108]. The lack of effect of diet on sex difference in the gut microbiome in laboratory mice may reflect the highly simplified diets they are fed and the artificial environment in which they are maintained[109]. Whether sex differences in the effects of diet on the gut microbiome in humans contributes to sex differences in diseases associated with dysbiosis, such as inflammatory bowel disease requires consideration. These data also imply that therapeutic approaches to treat diseases associated with dysbiosis may need to be different for males and females.

Effects of age and reproductive status

The age and reproductive status of an individual are also important determinants of sex-related differences in immune responses (FIG. 1). In the following section, we highlight some of the key immunological differences that are seen between the sexes at different stages of life.

In utero. Adverse fetal conditions may cause epigenetic adaptations leading to altered gene activity that can persist throughout life, including immunological programming[110]. Sex-differential developmental programming *in utero* results in sex differences in the local milieu and immune system development. Female fetuses have greater adaptability to intra-uterine stress than males. The placentas from premature (that is, born at <32 weeks gestation) male fetuses tend to be more chronically inflamed compared with those from female fetuses[111], providing females survival benefits from better cardiovascular stability and lower levels of circulating cytokines. Human male testes produce androgens from 10 weeks of gestation[112], leading to early development of androgen-dependent sex differences in immunity. Male neonates have higher cord blood IgE levels than females[113], a fetal product that may predict the development of atopy.

Birth to 5 years of age. At birth the fetus transitions from the placenta to the outside environment and is bombarded with new antigens. Multiple studies have investigated neonatal immunity using cord blood samples due to the availability of large blood volumes, but few have specifically looked for sex differences. Cord blood mononuclear cell reactivity to TLR agonists in full term infants and pre-term infants that were born at <33 weeks of gestation is not affected by sex in either group[114]. However, human male infants have higher monocyte and basophil counts compared to females up until 13 months of age, at least in high pathogen burden settings[115]. NK cell frequencies are higher in male than female children[25]. Infant males have greater pro-inflammatory responses than females following stimulation with either LPS or mitogens[116], all suggesting that males develop more robust innate immunity compared with females in early life.

Female cord blood contains higher numbers of $CD4^+$ T cells, higher CD4/CD8 T cell ratios and lower numbers of $CD8^+$ T cells and NK cells than cord blood from males, and these differences persist throughout childhood. By contrast, B cell numbers are comparable in male and female children[25,26]. The existence of these sex differences before puberty is often interpreted as evidence for genetic differences between the sexes. Neonatal castration experiments in rodents illustrate that the neonatal sex steroid milieu influences thymic development causing sex differences in the peripheral T cell compartment, including lower CD4/CD8 T cell ratios and higher NK T cell and $CD4^+CD25^+FOXP3^+$ T_{reg} cell numbers in neonatal males[117]. By contrast, there are no sex differences in human T_{reg} cell frequencies in Australian infants from birth to one year of age[118]. Nigerian female children (aged 5–12 years) have lower IgA levels, but equivalent IgG and IgM levels, compared with males[119].

Puberty. The multiple effects of sex steroids on immune cells play a prominent role in sex differences in inflammatory status during puberty. Although infant males may produce higher inflammatory responses than females, after puberty inflammatory responses are consistently higher in females than in males[120]. Females continue to have higher $CD4^+$ T cell counts and higher CD4/CD8 T cell ratios than males throughout adulthood[27,121], whereas males have higher numbers of T_{reg} cells[33]. Gene expression studies in mice show that post-pubertal females show increased expression of genes associated with the adaptive immune response (for example, immunoglobulin and B cell receptor genes), whereas males show increased expression of genes that are associated with innate responses (for example, serum amyloid A, haptoglobin and plasminogen activator inhibitor 2 as well as *Ccl9* and *Ccr1)*[122].

Because sex steroids profoundly affect the immune response, it is not surprising that hormonal changes during the female menstrual cycle underlie cyclical changes in immune function and exacerbation of diseases, with pronounced fluctuations in immune cell numbers and function occurring over the menstrual cycle, including increased numbers of T_{reg} cells when E2 concentrations are highest before ovulation[123].

Reproductive senescence. Women, worldwide, have a longer lifespan than males, with biological factors, including changes in sex steroid concentrations and X chromosome diploidy, having a significant role[124]. With age, concentrations of sex steroids decline rapidly for females and more gradually for males, paralleling a progressive functional decline in the immune system of both sexes[125]. One of the most well characterized attributes of an ageing immune system is an aberrant chronic low-grade pro-inflammatory state, thought to occur to a greater extent in females than males[35]. NK cell numbers increase with age, with the kinetics of this rise being greater for females than males[126]. Female menopause is associated with a decline in certain lymphocyte subsets, but elderly males experience a more rapid decline in numbers of B cells and various $CD4^+$ T cell subsets and show lower levels of T cell proliferation than females, along with a more modest increase in $CD4^+$ memory T cells following antigen re-exposure[34,126]. A population of B cells referred to as 'age-associated B cells' has been linked with the development of autoimmune diseases in mice and humans, and these cells are found in higher frequencies in aged female mice than in aged male mice[127].

Sex differences in the pathogenesis of diseases

Autoimmune diseases. Women represent ~80% of all cases of autoimmunity in the US[128]. Sex differences in the incidence of autoimmunity are most pronounced for Sjögren syndrome, systemic lupus erythematosus, thyroid diseases (such as Hashimoto thyroiditis and Graves disease), scleroderma, and myasthenia gravis, with significantly more women afflicted than men (FIG. 2). Although less common, there are some autoimmune diseases, including myocarditis and idiopathic pulmonary fibrosis, that show greater incidence in males than females, which is hypothesized to reflect higher T_H1 cell responses to self antigens during the acute phase of these autoimmune diseases[129].

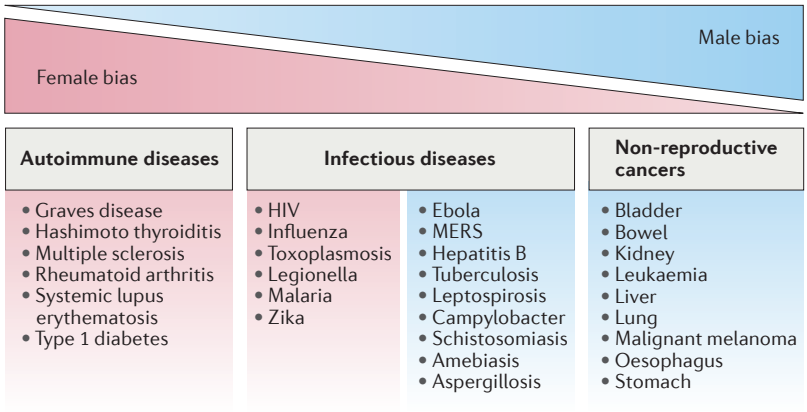


Figure 2 | **Sex bias in infectious diseases, inflammatory diseases and cancers.** At the extremes, males and females show robust differences in their susceptibility to autoimmunity and cancers. Generally, females show increased susceptibility to autoimmune disease development and males show increased susceptibility to non-reproductive malignant cancers. Although at a less pronounced magnitude, sex differences are also seen in susceptibility to various infectious diseases. Reproductive status, including pregnancy, as well as immune-mediated pathology contributes to female-biased infectious diseases, whereas pathogen-associated damage, including delayed clearance, is associated with male-biased infectious diseases. MERS, Middle East respiratory syndrome.

Animal models, including the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis and the nonobese diabetic (NOD) mouse model of spontaneous type 1 diabetes, have been used to characterize the immunological and hormonal causes of sex differences in autoimmune diseases. The incidence and severity of autoimmune disease in NOD mice and in EAE models is higher in female than in male mice. Castration of males increases, whereas ovariectomy of females decreases, the incidence of autoimmune diseases in mouse models[130]. In EAE mice and in PBMCs from patients with multiple sclerosis, females show greater activation of T_H1 cells and increased levels of IFNγ production, whereas males exhibit greater T_H17 cell responses owing to androgen receptor regulation of PPARα expression in T cells[32]. In female mice with EAE and women with severe forms of multiple sclerosis, administration of high doses of oestrogens suppress cell-mediated immune responses and relieves disease symptoms[130,131]. In men with multiple sclerosis, topical treatment with a gel containing testosterone slows brain atrophy and shifts peripheral immune responses by reducing the numbers of $CD4^+$ T cells and PBMC production of IL-2 and increasing numbers of NK cells and PBMC production of TGFβ[132] Thus, administration of hormone supplements to patients with autoimmune diseases may have novel therapeutic applications.

Malignancy. Sex is an important factor in the pathogenesis and prognosis of many cancers that occur outside of the reproductive tract (FIG. 2). For the majority of cancers throughout life, the risk of malignancy is higher for males[133]. Males have an almost twofold greater risk of mortality from all malignant cancers than do females[134], with sex-differential outcomes being greatest for larynx, oesophagus, bladder and lung cancers[134]. This male-biased mortality is hypothesized to reflect differences in cancer aetiology[134], including sex differences in viral infection, immune function, hormonal regulation, gene expression, sex chromosome complement, oxidative damage, autophagy, or a combination of factors[134,135]. Even treatments for cancers show sex-specific outcomes. Immune checkpoint inhibitors are revolutionizing cancer treatment, and some treatments, including programmed cell death 1 ligand 1 (PDL1)-specific monoclonal antibodies, appear to be more efficacious in female patients compared with male patients with melanoma[136]. The sex-differential effects of cancer chemotherapies with immunomodulatory properties and cancer vaccines require dedicated research.

Infectious diseases. The sexes differ in the severity, prevalence, and pathogenesis of infections caused by viruses, bacteria, parasites and fungi, with males generally more susceptible to these infections than females[137] (FIG. 2). These differences are observed for infectious diseases acquired via multiple routes such as person-to-person, vector-borne, blood-borne, and food and water borne[124], with sex differences in immunity playing a major role[138]. Newborn males are more vulnerable to infections and death than females[139]. In several developing countries, school-age male children have higher rates of protozoan infections (such as, malaria caused by *Plasmodium falciparum*, visceral leishmaniasis and *Entameba histolytica* induced amoebic liver abscess), trematode infections (such as, schistosomiasis caused by *Schistosoma mansoni*) and nematode infections (such as those with *Necator americanus*, *Toxocara* spp. and *Wuchereria bancrofti*)[140]. Among adults, untreated HIV-1-infected women have greater $CD8^+$ T cell activation than men when adjusted for viral load and over 40% less circulating HIV RNA than men; however, when matched with men by HIV RNA load, woman have a 1.6-fold higher risk of developing AIDS[141]. Although exposure to influenza A viruses is often higher in men, fatality following exposure to pathogenic influenza A viruses is higher in women; by contrast, the prevalence of serum hepatitis B virus (HBV) surface antigen, HBV DNA titers and development of hepatocellular carcinoma is higher in men than women. In most countries, tuberculosis notification is twice as high for men than women. Clinical cryptococcosis is 10 times higher for immunocompromised men than women. Heightened immunity to pathogens among females contributes to lower intensity (that is viral load within an individual) and prevalence (that is number of infected individuals within a population) of many infections for females than males, but it may increase disease symptoms and severity among females compared with males[137].

Vaccines. Sex differences have been described in immunity to multiple vaccines, including both inactivated vaccines (such as vaccines against brucellosis, diphtheria, hepatitis A, hepatitis B, herpes simplex virus-2 infection (genital herpes), influenza, meningococcal

Table 4 | **Sex differences in responses to vaccines in humans**

Target group	Vaccine	Sex difference in Immune response	Sex difference in adverse reactions	Age (years)
Children	Hepatitis B	Greater in females	Not defined	<12
	Diphtheria	Greater in females	Not defined	<2
	Pertussis	Greater in females	Not defined	<2
	Pneumococcal	Greater in females	Not defined	6–9
	Rabies	Greater in females	Not defined	6–9
	Measles	Greater in females or equivalent in both sexes	Increased in females	<3
	RTS,S vaccine against malaria	Greater in females	Increased in females	<2
	Human papillomavirus	Greater in females	Increased in females	5–17
Adults	Influenza	Greater in females	Increased in females	18–49
	Hepatitis B	Greater in females	Increased in females	>18
	Herpes virus	Greater in females	Not defined	>18
	Yellow fever	Greater in females	Increased in females	>18
	Rabies	Greater in females	Not defined	>18
	Smallpox	Greater in females	Not defined	>18
Aged adults	Influenza	Greater in females	Increased in females	>65
	Td/Tdap	Greater in males	Increased in females	>65
	Pneumococcal	Greater in males	Increased in females	>65
	Shingles	Not defined	Increased in females	>65

meningitis, pneumococcal disease (using pneumococcal polysaccharide), rabies and tetanus) and live vaccines (such as those against measles, rubella, smallpox, Venezuelan equine encephalitis, and yellow fever), in both children and adults[142]. The biological differences between the sexes is a major source of variation in the immune response to vaccination[11,124] (TABLE 4). Antibody responses to bacterial and viral vaccines are often higher in females than males (TABLE 4). This could mean that the effective vaccine dose is lower for females than for males. For example, in dose response studies with the inactivated influenza vaccine, human females vaccinated with a half dose influenza vaccine achieved equivalent antibody titres to males vaccinated with full dose vaccine[143]. Females consistently report more frequent and severe local and systemic reactions to viral and bacterial vaccines than males, at least among young and ageing adults[11,124,142], reflecting either a reporting bias or greater inflammatory responses among females than males[11]. Whether sex differences in immune responses to vaccines are caused by genetic, hormonal and environmental factors, or a combination, requires consideration.

Conclusions and perspective

The basis for personalized medicine is that unique aspects of our biology, including our immune responses, will define novel targets for more effective prevention and treatment of immune-related diseases. In this Review, we provide evidence that sex is one variable that influences innate and adaptive immune responses, resulting in sex-specific outcomes from infectious and autoimmune diseases, malignancies, and vaccines. If the long-term goal of personalizing treatments for immune-mediated diseases is effective treatment for all individuals, then will we ultimately treat males and females differently in an effort to protect them equally? Future studies must identify the precise factors mediating sex differences in the immune responses, knowing that this will probably reflect complex interactions among hormones, genes and our environment (both biotic and abiotic). Sex-based differences in the activity of the innate and adaptive immune responses likely have evolved through a process of convergent evolution, in which the fundamental mechanisms that underlie increased survival and reproductive success have sex-specific effects on immune function.

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