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Trends in Immunology

Feature Review

Genetic and hormonal mechanisms underlying sex-specific immune responses in tuberculosis

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Tuberculosis (TB), the world's deadliest bacterial infection, afflicts more human males than females, with a male/female (M/F) ratio of 1.7. Sex disparities in TB prevalence, pathophysiology, and clinical manifestations are widely reported, but the underlying biological mechanisms remain largely undefined. This review assesses epidemiological data on sex disparity in TB, as well as possible underlying hormonal and genetic mechanisms that might differentially modulate innate and adaptive immune responses in males and females, leading to sex differences in disease susceptibility. We consider whether this sex disparity can be extended to the efficacy of vaccines and discuss novel animal models which may offer mechanistic insights. A better understanding of the biological factors underpinning sex-related immune responses in TB may enable sex-specific personalized therapies for TB.

Sex inequality in World Health Organization TB data

Global epidemiological data suggest that TB case notification rates among human males are consistently higher than in females, and that this phenomenon has been sustained for many decades [1]. Despite a 18% reduction in case notifications between 2019 and 2020 as a result of the coronavirus disease 2019 (COVID-19) pandemic, the global M/F ratio of TB incidence in HIV-1-negative adults varied from 1.12 in the Eastern Mediterranean Region to >2 in the Americas and the Western Pacific Region [1]. Data indicating that males are at a higher risk of developing TB than females across geographical regions are compelling [2]; however, sex differences in TB susceptibility remain understudied, and only a few underlying immune mechanisms have been elucidated. Given the importance of sex equality combined with the World Health Organization (WHO)-endorsed End TB Strategy goal to eradicate the TB epidemic by 2035 [3], this striking sex-based difference warrants new research. This review describes our current understanding of mechanisms that may account for this sex-based difference in TB pathophysiology with a focus on immune regulation – hormonal and genetic – that may be at play.

Biological intricacy or epidemiologic error?

Demographic factors affecting sex bias in TB

Sex bias in TB is still an intriguing conundrum. One potential explanation for the male sex bias is that it may represent an epidemiological artifact resulting from various socioeconomic, geographical, and cultural factors. A common hypothesis has been that females do not have equal access to healthcare [4,5]. The male bias, however, also occurs in developed countries where barriers to healthcare for females, including TB diagnosis, are reduced [6]. Regarding biased reporting, a study found that male TB prevalence was twofold higher than for females, and from 1993 to 2006, diagnosis took 1.5-fold longer in males in low- and middle-income countries [2]. These findings concurred with other studies suggesting that pulmonary TB in females is more likely to be diagnosed than in males [7].

Multiple lines of evidence from humans also support a biological basis for this sex difference. First, the 1929 Lübeck disaster showed convincing evidence for a direct biological basis for male sex

Significance

Tuberculosis (TB) continues to remain one of the world's most important medical challenges with a significant male bias. Elucidating the underlying mechanisms that mediate differences in immune responses to *Mycobacterium tuberculosis* in males and females will not only enlighten our understanding of TB pathogenesis but also aid in developing novel sex-specific vaccines and therapies.

Highlights

Human adult males are 1.7-fold more likely than females to develop active tuberculosis (TB) disease. Recent studies indicate that reporting biases and differential healthcare access do not fully explain the higher TB incidence in males worldwide.

Sex steroid hormones, sex chromosome-encoded genes, and miRNAs differentially modulate innate and adaptive immune responses to TB in males and females, both in humans and in animal models of TB.

Research addressing the biological bases for the male bias is likely to provide novel insights into the pathogenesis of active TB disease and latent TB infection.

Understanding the immunological basis for the male bias might enable the development of precision medicine interventions for TB, including host-directed therapies that are sex-specific.

Dissecting the molecular and immunological mechanisms of the male bias in TB will benefit from improved animal models and sex-linked biomarkers that can predict the risk of developing active disease.

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bias in young children. In this tragic event, 251 neonates were accidentally inoculated with Bacillus Calmette–Guérin (BCG) contaminated with virulent *Mycobacterium tuberculosis* (Mtb) [8]. In the aftermath investigation, death was concluded to be due to TB, which was significantly more frequent in male (50/137, 36.5%) than female children (27/114, 23.7%), with an odds ratio of 1.67 [8]. Second, in a study of 74 castrated males and 170 age-matched human controls from the same era, castrated males were statistically significantly less likely to die from TB (6/74, 8.1%) than gonadal males (35/170, 20.6%) [9]. Third, a recent retrospective cohort study and parallel meta-analysis of the past 10 years of published reports on the clinical outcomes of TB revealed that male patients had higher mortality, and higher sputum culture and smear positivity rates, during and after TB treatment than females [10]. Fourth, studies examining immune biomarkers for TB found a higher interferon gamma (IFN-γ) response to Mtb antigens CFP-10 and ESAT-6, and lower IgM titers in males, while a strong tuberculin reaction was observed in smear- and culture-positive female patients [11]. In addition, male contacts of pulmonary TB patients who did not develop TB exhibited significantly larger positive tuberculin test reactions than the general cut-off – an observation suggesting that sex-specific and age-matched cut-offs for defining tuberculin positivity might be warranted [11]. Of note, pulmonary TB produces a spectrum of abnormalities, some of which (e.g., cavitary disease) show equal distribution among the sexes. In addition, lower lung field TB may be more prominent in women, requiring more risk-stratification tools to evaluate sex differences [12,13].

Does the sex bias hold up in immunocompromised patients?

Given the substantial evidence that the male sex bias has a biological basis, a follow-up question is whether the sex bias is evident even in immunocompromised patients. The male sex bias has been studied in TB/HIV-1 coinfected patients since HIV-1 infection is one of the highest risk factors for TB [14]. Of 128 Mtb/HIV-1 coinfected patients in Colombia, 79.7% were men [15]. In a similar retrospective study of 666 drug-resistant TB/HIV-1 coinfected patients in Uganda, 60.2% were men, with a median age of 37 years. In addition, mortality following treatment failure was higher in men (25.7%) than in women (18.5%, $P = 0.04$) [16]. Table 1 provides a summary of studies that have addressed the male sex bias in TB/HIV-1 coinfection.

Is there a sex bias in latent TB infection?

Sex disparity in the prevalence of **latent TB infection (LTBI)** (see Glossary) is not as well studied as **active TB** (**AT**), and existing studies reveal conflicting findings. Despite the global AT M/F ratio of 1.7, many regional surveys have not found a sex bias in human LTBI [17–19]. For example, a study on high-risk individuals revealed that the proportion of LTBI was higher in males than in females (32.6% vs. 25.2%, $P = 0.010$). However, following multivariate analysis after considering

Table 1. Male predominance in TB/HIV-1 coinfection[a]

Type	Setting	Number	M/F ratio	*P value*, HR, or AHR	Refs
New TB incidence among HIV-1$^+$ subjects					
Retrospective	South Africa	2423	2.16	<0.001 AHR	[127]
Prospective	South Africa	2778	1.46	<0.05 HR[b]	[128]
Retrospective	South Africa	240	1.45	NA	[129]
TB registries in a large region (with data on sex and HIV-1 status)					
Retrospective	Zimbabwe	13 802	1.38	0.017	[130]
Retrospective	Ethiopia	2252	1.19	NA	[131]

[a]Abbreviations: AHR, adjusted HR; HR, hazard ratio; NA, not available.
[b]The AHR was 1.22, $P = 0.08$.

age, smoking status, and clinical factors, male sex was not found to be an independent factor for LTBI [17]. One interpretation of the available data is that, although females and males may have a similar risk of developing LTBI, males with LTBI might progress to AT more readily. Stated another way, male immunological containment responses during LTBI might fail to provide the same degree of Mtb containment as those in females, although this remains conjectural. Of note, a serological survey using multivariate analysis of 383 Canadian subjects with AT or LTBI found that antibody titers against the 14 kDa antigen of Mtb were significantly associated with LTBI and female sex, suggesting their potential use in identifying individuals at high risk of LTBI reactivation [20]. Thus, biological factors clearly play important roles, and research addressing the biologic bases for the male bias is likely to unmask novel insights into the pathogenesis of human AT and LTBI. Altogether, under-diagnosis and under-reporting in females presumably may affect the epidemiology data in some settings, but these alone cannot account for the global sex bias towards males in TB, which further raises the question as to why males are more susceptible to TB than females.

Why are males more likely to develop AT?

Two different hypotheses – physiological versus behavioral – have been proposed to explain the phenomenon of sexual inequality in infectious diseases [21]. The latter posits that the biased infection rates are primarily caused by sex-specific differences in pathogen exposure. By contrast, the physiological hypothesis is that sex hormones together with genetic effectors encoded on the sex chromosomes are likely determinants of immunity and disease susceptibility [21]. Indeed, a survey of case notifications and age-stratified M/F prevalence pattern in Brazil suggests that TB male sex bias is minimal during childhood and becomes prominent only after puberty – an observation indirectly implicating sex hormones as putative key mediators of the TB sex bias (Figure 1, Key figure) [21,22]. A study of TB incidence in Tuscany showed that the male proportion ranged from 51.4% in children (0–14 years) and adolescents (15–18 years), but escalated to 60.9% post-puberty (≥18 years), suggesting that male TB incidence rises after sexual maturation [23]. Importantly, the female protective effect from TB is observed in both pre- and post-menopausal phases; although the incidence of female TB increases in post-menopausal elderly age groups compared to adults, it does so at a lower rate than in males, suggesting that aging perhaps intensifies pulmonary TB incidence in males [24]. A recent study from Shandong Province in China from 2005 to 2017 showed that, compared to the non-elderly, pulmonary TB incidence was higher in the elderly population (aged ≥60 years) and, of the 77 192 elderly TB cases, 76.8% occurred among males, indicating an even higher M/F ratio of TB incidence among the elderly population [24]. Although further investigations will be necessary to ascertain if the observed resistance in females is modulated hormonally or has a genetic basis, sex- and age-specific methods for the prevention and control of pulmonary TB may be warranted.

Animal models reveal a physiological basis for TB sex differences

Various animal models have been employed to assess physiological factors in TB sex differences. A 1947 study showed that castrated guinea pigs of both sexes exhibited increased survival upon TB infection, and no male versus female differences were observed [25]. However, two recent murine studies have now demonstrated that TB disease severity is greater in male mice than in females [26,27]. Specifically, gonadectomized (Gdx) and sham-operated female and male BALB/c mice were assessed for Mtb susceptibility. Sham-operated male mice showed more rapid lethality and higher organ bacterial burdens than Gdx males or sham-operated females [26]. Compared to non-castrated males, sham-operated female and Gdx male lung tissues showed significantly higher mRNA expression of proinflammatory cytokines tumor necrosis factor alpha (TNF-α), IFN-γ, interleukin (IL)-12, and IL-17, as well as iNOS, suggesting a more robust immunological response in animals lacking testes [26]. A following study observed the same

Glossary

Active tuberculosis (AT): a multiorgan disease caused by *Mycobacterium tuberculosis* (Mtb) primary infection or reactivation of its latent infection (~90% of the cases) in an immunocompromised background. Unlike the asymptomatic latent form, AT is highly contagious and causes symptoms depending on whether it is pulmonary or extrapulmonary.

Autophagy: conserved lysosome-dependent degradation of a cell to remove dysfunctional components. It regulates inflammation and generates cell-autonomous defense against intracellular pathogens such as Mtb. Autophagy is often looked upon as an adaptive stress response for survival; however, in some diseases it appears to promote cell death and morbidity.

B cell follicles: a complex network of colocalized cells comprising B cells, follicular dendritic cells, T cell subsets, and adjoining macrophages. These cells reside in primary and secondary follicles of lung, spleen, and lymph nodes, and participate in T cell-dependent antibody responses mediated by follicular B cells.

Bone marrow-derived macrophages (BMDMs): cells extracted from mammalian bone marrow cells and differentiated under *ex vivo* settings into mature macrophages by stimulation with growth factors and signaling molecules.

Class-switch recombination (CSR): a complex genetic process in B cells in which DNA-rearrangement reactions of immunoglobulin heavy chains cause a switch from IgM antibody production to IgG, IgE, or IgA.

C3HeB/FeJ 'Kramnik' mice: transgenic C3H mice that carry a normal allele at the *Tlr4* locus but that are homozygous for *Pde6b*^{rd1}. C3HeB/FeJ inbred mice are extremely susceptible to Mtb and, unlike BALB/c and C57BL/6 mice, develop necrotic lung lesions following aerosol challenge.

Genetic imprinting: the differential epigenetic marking of paternally and maternally inherited alleles of specific genes or chromosome regions during gametogenesis, leading to monoallelic expression depending on parental origin. Female mammals possess two X chromosomes, one from each parent, whereas in males the single X chromosome is invariably of maternal origin.

Genotype–tissue expression project (GTEx): an open-access public

male-skewed increased lethality and higher organ burdens compared to females in the C57BL/6 mouse background upon aerosol infection with Mtb [27]. However, in contrast to the previous study [24], infected male C57BL/6 mice lung homogenates contained significantly higher proinflammatory cytokines such as IL-1α, IL-1β, IFN-γ, and IL-6, and wider range of chemokines including RANTES (regulated upon activation, normal T cell expressed and presumably secreted), MIP-3α (macrophage inflammatory protein-3 alpha), and CXCL1 (chemokine ligand 1) over time, than female mice [27]; this re-emphasizes the notion that proinflammatory cytokines are essential for the containment of Mtb infection, but they may become detrimental when produced in a dysregulated manner. Lymphoid aggregates rich in **B cell follicles** are archetypal for both mouse and human lung granulomas, and are essential for immune control during the containment of Mtb infection [28]. In this milieu, the development of smaller B cell follicles in males over time, compared to Mtb-infected female C57BL/6 mice, may contribute to reduced resistance and higher TB susceptibility in males [29]. In addition, the expression of CXCL13 and CCL19, chemokines involved in the homing of lymphocytes and myeloid cells to infected areas of the lung, was significantly elevated in females compared to males, consistent with the impairment of B cell follicle formation in males [29]. In addition, BCG-vaccinated females were better protected from TB than males in mouse models (Box 1) [30]. Compared to males, similar protection from disease progression in females has also been observed for *Mycobacterium bovis* infection in red deer, and non-tuberculous mycobacterial (NTM) infections in mice [31–35]. Of note, in humans, the clinically important NTM pulmonary disease syndromes caused by *Mycobacterium avium* and *Mycobacterium abscessus* are more common in females than in males, possibly due to related age- and sex-specific risk factors (Box 2) [36–38]. These discrepancies suggest that host immune responses to Mtb are different from those evoked by NTMs, leading to differing sex ratios. Together, these animal studies clearly indicate a physiological basis for increased male susceptibility to TB, and reveal how gonads can influence the course of experimental TB in both the sexes to different degrees.

Sex hormones as modulators of anti-TB immune responses

During pulmonary TB, gene regulation and immune cell recruitment in the lung may be modulated by sex hormones (Box 3 and Figure 1) [39]. Using the human **genotype–tissue expression project (GTEx)** data from non-diseased, healthy tissues of 838 individuals (557 males and 281 females), 13 294 genes were identified that showed sex-based differential expression across 44 different tissues [40]. Sex-dependent expression of autosomal genes suggested regulation either via sex hormones and their receptors or stemming from sex-dependent epigenetic changes influencing transcription [40]. Accordingly, the promoters of a large number of sex-biased genes harboring **hormone-response elements (HREs)** are recognized by transcription factors (TFs) such as the estrogen receptor-α (ERα), glucocorticoid receptor (GR), as well as two X-linked TFs – the androgen receptor (AR) and ELK1 [40]. Androgen-response elements (AREs) and estrogen-response elements (EREs) are present in the promoters of several innate immunity genes [41].

Effect on innate immune responses

Innate immune cells such as macrophages, neutrophils, and natural killer (NK) cells that mediate type I immune responses play important roles in determining the outcome of disease following Mtb infection because they influence the early inflammatory response, cytokine secretion, and the recruitment and activation of other innate and adaptive immune cells, eventually leading to either containment of infection or disease progression [42]. Because these cells express sex hormone receptors, steroid signaling pathways mediated by them could induce differing functional responses between males and females during early host interactions with Mtb (Figure 1) [43]. Lung macrophages are the first cells to encounter Mtb during infection, and they mount multiple defense strategies to contain the infection, including phagocytosis, induction of antimicrobial

domain for comprehensive analysis of tissue-specific gene expression and regulation (https://gtexportal.org/home). GTEx helps to correlate genotypes and differential gene expression in the context of genetic or sex hormone-related factors.

Hormone-response elements (HREs): short DNA sequences in a gene promoter to which specific steroid hormone receptor complexes bind and regulate transcription.

Host-directed therapy: in the context of infectious diseases, refers to treating infections with agents that alter host responses rather than killing or inhibiting microbial proliferations.

Hyper-IgM syndrome (HIGM): rare genetic defects with an inability to switch from IgM antibody production to the IgG, IgA, or IgE types. HIGM patients are susceptible to recurrent and severe infections, risk of cancer, and opportunistic infections.

Latent TB infection (LTBI): asymptomatic Mtb infection with immunological evidence of responsiveness to Mtb antigens. Typical immunological diagnostics for LTBI are tuberculin skin test or IFN-γ release assay (IGRA) positivity.

Mendelian susceptibility to mycobacterial disease (MSMD): a rare genetic disorder associated with susceptibility to infections caused by weakly virulent mycobacteria such as Bacillus Calmette–Guérin (BCG) and non-tuberculous mycobacteria (NTMs). Defects in nine genes – seven autosomal (*STAT1*, *IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *ISG15*, and *IRF8*) and two X-linked (*NEMO* and *CYBB*) – are known to cause MSMD in humans.

PD-1/PD-L1 immune checkpoint: PD-L1 is a transmembrane protein involved in suppressing the adaptive arm of immunity; PD-1 is a checkpoint receptor abundantly expressed by activated T cells, B cells, dendritic cells, and natural killer (NK) cells. Binding of PD-L1 to PD1 triggers a series of pathways leading to inhibition of T cell proliferation and T_{reg} cell apoptosis.

Regulatory T cells (T_{reg}s): a subpopulation of $CD4^+$ T cells with immunosuppressive functions involved in sustaining homeostasis and self-tolerance.

Sex-determining region on chromosome Y (SRY): a SOX gene family transcription factor ('testis-determining factor') encoded by the Y chromosome; SRY is required for the development of male gonads. In

effectors such as nitric oxide (NO), cytokine production, and induction of **autophagy** [42]. Compared to males, macrophages from adult female C57BL/6 mice show greater phagocytosis of zymosan particles and NADPH oxidase-mediated killing of group B streptococci *in vitro* [44]. Human and mouse macrophages express ERα, the intracellular estrogen receptor (ER); however, macrophage phagocytosis is impaired in **bone marrow-derived macrophages (BMDMs)** from C57BL/6 mice that carry a myeloid-specific ERα deletion [45]. This study showed that ERα is important for maintaining other macrophage functions including oxidative metabolism, as well as responses to lipopolysaccharide (LPS) and proinflammatory fatty acids [45]. Neutrophils play dual and opposing roles during Mtb infection: during early infection, neutrophils phagocytose Mtb and also generate NADPH oxidase-mediated reactive oxygen species (ROS), as well as peptides such as cathelicidin LL-37 and lipocalin 2 – all of which are antimicrobial, as shown in studies examining Mtb growth in human peripheral blood cells [46–49]. Studies using zebrafish show that neutrophils exert a protective effect through oxidative killing of the mycobacteria phagocytosed from dead granuloma macrophages [50]. Human neutrophils express ERα and ERβ [51]. Phagocytosis of heat-killed *Staphylococcus aureus* by neutrophils derived from the peripheral

mammals, *SRY/Sry* presence leads to development of testis; in its absence, ovaries develop.

Toll-like receptors (TLRs): innate immune receptors expressed by macrophages, dendritic cells, NK cells, and T and B cells; TLRs recognize pathogen-associated molecular patterns and activate innate immune cell responses.

X-chromosome inactivation (XCI): also known as lyonization or Barr body formation, XCI is a normal physiological process in females by which one copy of the X chromosome is inactivated and becomes transcriptionally inactive heterochromatin.

Key figure

Sex hormone effects that may contribute to the male bias in immune responses to tuberculosis (TB)

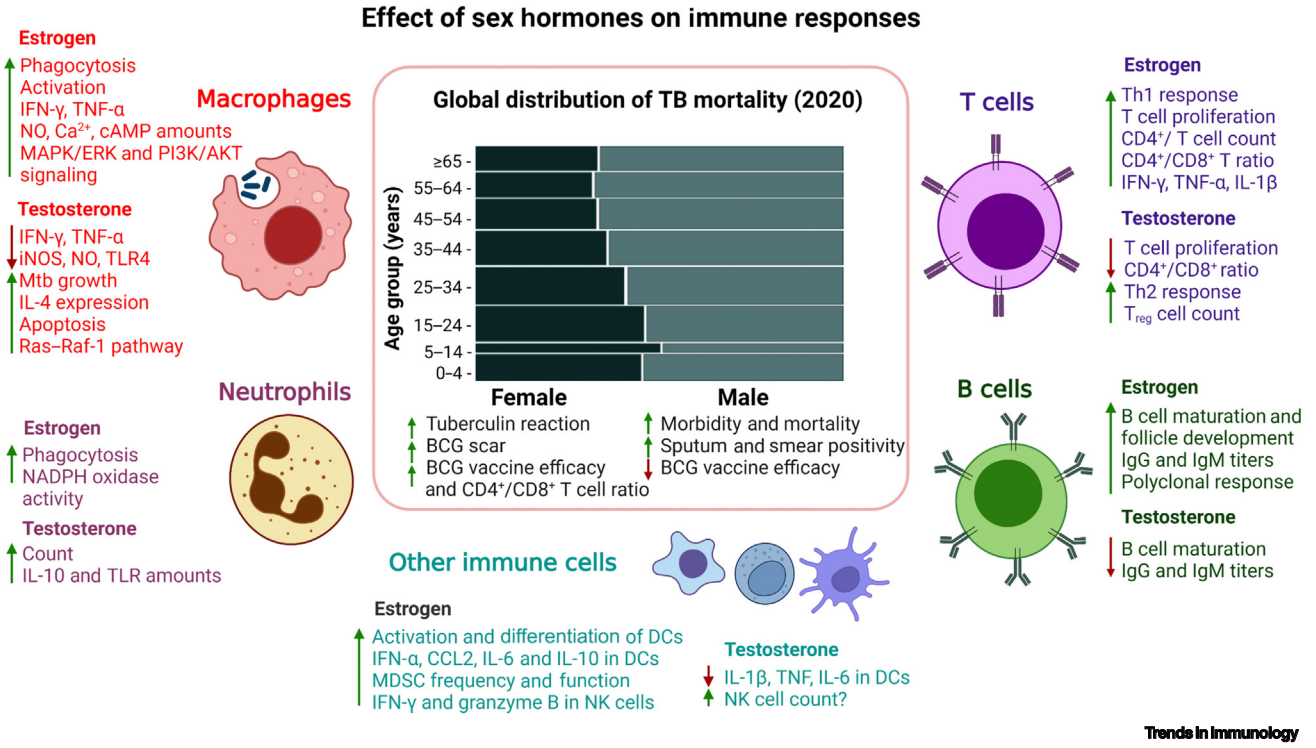

Figure 1. Effects of estrogen and testosterone on human or mice immune cells that are known to play a key role in anti-TB immunity [26,68]. (Center panel) Global sex- and age-based distribution of TB mortality in 2020 (adapted from the 2021 WHO TB report) [1]. Schematic representation, images not to scale. Abbreviations: BCG, Bacillus Calmette–Guérin; CCL2, chemokine (C-C motif) ligand 2; DCs, dendritic cells, IFN-γ, interferon gamma; IL, interleukin; iNOS, nitric oxide synthase; MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2; MDSCs, myeloid-derived suppressor cells; Mtb, *Mycobacterium tuberculosis;* NK cells, natural killer cells; NO, nitric oxide; PI3K/AKT, phosphatidylinositol 3-kinase/protein kinase B; Th, T helper cell; TLR-4, Toll-like receptor 4, TNF-α, tumor necrosis factor alpha; T_{reg}, regulatory T cell; WHO, World Health Organization.

Box 1. Sex differences in BCG vaccine efficacy

BCG is used to vaccinate neonates in most nations for TB prevention [132]. It protects children from disseminated TB during the first decade of life, but has little efficacy in preventing adult forms of TB [133]. The magnitude of the BCG response as measured either by scar size or the tuberculin skin test reaction shows a strong correlation with higher overall childhood survival among female versus male children [134]. Post-BCG vaccination, a sex-dependent reactivity to TLR2, 4, 7, and 8 ligands has been observed, with significantly higher IL-10 and IL-17 responses in males than in females [135]. Several recent studies have identified sex differences in immunological responses to BCG in adult volunteers receiving it as an experimental vaccine. Using a proteome platform to assess systemic inflammatory markers, BCG vaccination of adults enhanced cytokine responses (IL-1β, IL-6, and TNF-α) to Mtb restimulation but reduced systemic inflammation to a significantly greater degree in males than females, as evidenced from significantly lower concentrations of circulating inflammatory proteins, including CXCL1, CXCL5, CXCL6, MCP-4, CD40, and CASP-8, at 3 months after vaccination [136].

In a cohort of 100 healthy individuals, BCG-vaccinated females showed higher $CD4^+/CD8^+$ ratios, and their peripheral blood mononuclear cells controlled Mtb growth better than males [137]. Using the TB-susceptible 129 S2 mouse model, a recent study revealed a lower lung Mtb burden and hence better protection in BCG-vaccinated female mice than in males, reiterating sex differences in BCG vaccination responses [30]. Further, 4 weeks after Mtb infection, the lungs of vaccinated male mice showed a significantly higher number of neutrophils, inflammatory monocytes, and $CD11b^+GR\text{-}1^-$ monocytes/macrophages, and a higher myeloid cell-to-T cell ratio, than females [30]. Conversely, female mice tended to have an overall increased number of total and $CD4^+$ T cells in lungs compared to males, which was consistent with the well-known effect of androgens on abbreviating T cell responses [30]. In addition, T_{reg} cell expansion following human BCG vaccination has been postulated to correlate with relatively poor efficacy of BCG, and human males possess more T_{reg} cells than females [138–140]. Therefore, it is possible that T_{reg} cells might contribute to modulating BCG efficacy in a sex-dependent manner, although this remains to be rigorously tested.

blood of healthy human females of reproductive age has been found to be higher than by cells from age-matched males [52]. Moreover, neutrophils from granulomas of Mtb-infected macaques express pro- (IFN-γ, TNF-α) and anti-inflammatory (IL-4, and IL-10) cytokines, suggesting that they modulate immune homeostasis in the granuloma microenvironment [53]. During the later stages of Mtb infection, excess neutrophil infiltration leads to increased severity of inflammation, and is associated with pathology in active human TB [54]. Although not evaluated in TB, neutrophil recruitment in prostate inflammation induced by bacterial infection is higher in testosterone-treated rats, and is associated with infiltration of inefficient 'N2-like' neutrophils, as well as with the expression of IL-10, thus prolonging inflammation and tissue damage [55]. Type 1 invariant natural killer T (iNKT) cells also are important innate immune cells that contribute to early antimycobacterial responses via the production of IFN-γ and GM-CSF [56,57]. *In vitro* and *in vivo* challenge with the specific iNKT cell ligand α-galactosylceramide (αGalCer) induces higher IFN-γ responses in female versus male C57BL/6 mice, and also *in vitro,* via exposure of iNKT cells to estradiol, but not in ovariectomized females [58]. Inhibition of IFN-γ secretion by testosterone has also been observed in a mouse model of amoebic liver abscess, a parasitic infection with a strong male bias in which disease control is influenced by NKT cells [59]. The differential roles these cells might play in Mtb infection in males and females remain to be elucidated. However, because early events dictate clinical outcomes in TB, sex hormones may play an important role in modulating key initial responses mediated by innate immune cells following Mtb infection.

The MAPK/ERK pathway is essential for the secretion of TNF-α, IL-8, and monocyte chemotactic protein-1 (MCP-1), as revealed by analysis of ERK1/2 and p38 kinases and quantitation of cytokines in culture supernatants following Mtb infection of human peripheral blood monocytes in the presence of specific inhibitors of p38 and MAPK-1 [60,61]. Mtb infection of primary human macrophages also suppresses the PI3K/AKT/mTORC1 pathway that negatively regulates MMP-1 – a primary mediator of tissue destruction and disease spread, as shown by gene expression and MMP-1 secretion [62]. To impede phagosome–lysosome fusion and promote intracellular survival, Mtb suppresses calcium signaling in human macrophages [63]. In this milieu, estrogen signaling via ERs localized on the inner cell membrane is associated with increased intracellular NO (antimycobacterial) and Ca^{2+} concentrations, activation of cAMP

Box 2. Non-tuberculous mycobacterial (NTM) infections: more common in human females than males

To date, over 170 naturally occurring NTM species are known, but pulmonary disease (PD) caused by *M. avium* complex (MAC), *Mycobacterium kansasii*, or *M. abscessus* is the most common [36]. NTMs may also cause cutaneous infections and lymphadenitis, although less frequently. Demographically, the incidence rate of NTM-PD is growing each year, and many studies across different countries show that NTM-PD is more common in adult females than males by a margin of 1.1–1.6-fold [36–38]. In particular, NTM-PDs have higher prevalence among elderly post-menopausal women than men, and, in contrast to TB, underlying chronic lung disease is a key risk factor [38]. Typical underlying risk factors for NTM-PD include chronic obstructive pulmonary disease (COPD), non-cystic fibrosis bronchiectasis (NCFB), and primary ciliary dyskinesia. Some body morphotypes (pectus excavatum or scoliosis) and slender body habitus are also risk factors, particularly among adult females [38]. Among the younger population, cystic fibrosis is a strong risk factor for NTM infection, but in this population, males and females are at equal risk [141]. These observations suggest that post-menopausal sex hormone concentrations, adipocytokine signaling, low-fat, and connective tissue disorders might contribute to the female bias in NTM-PD. In addition, host immune responses to Mtb could be different from those evoked by NTM, leading to differing sex ratios. However, experimental studies in murine models have not successfully recapitulated the clear-cut human female sex bias for NTM diseases, perhaps owing to the paucity of animal models that mimic the underlying lung diseases which predispose humans.

In multiple mice variants, gonadal males are more susceptible to *Mycobacterium marinum* than females and castrated males, whereas testosterone treatment increased the infection susceptibility of gonadal females [33]. Similarly, a male sex bias was observed in mice to *Mycobacterium intracellulare* and *Mycobacterium lepraemurium* infections [32,35]. After *M. avium* challenge, ovariectomized mice exhibited increased lung bacillary burden compared to sham-operated controls, and the effect was reversed upon exogenous estradiol treatment [34]. Because of the rising rates of NTM disease and the need for improved therapies, considerable attention is now being devoted to developing better animal models for NTM infections [142].

synthesis, and stimulation of the MAPK/ERK and PI3K/AKT signaling cascades [64]. Hence, estrogen signaling through intracellular ERs may play a protective role against TB. In contrast to the impact of estrogen signaling cascades to elevate cytokine production, testosterone induces apoptosis (thought to be host protective in TB) in mouse BMDMs via the Fas/FasL death-inducing signaling pathway and by activating caspase-3, caspase-8, and poly(ADP-ribose) polymerase (PARP) [65]. Sex hormones might therefore differentially activate intracellular signaling pathways in Mtb-infected macrophages that can determine the course of disease.

Box 3. Sex hormones and TB

Sex steroids such as estrogens, progesterone, testosterone, and dihydrotestosterone (DHT) vary extensively between sexes. Estrogen exists in three different isoforms: estrone (E1), estradiol (E2), and estriol (E3). Estradiol predominates in the sera of menstruating females (100–600 pg/ml), whereas the E1 isoform dominates in post-menopausal women when estradiol concentrations fall to levels similar to, or lower than, those of age-matched males (5–20 pg/ml). Female pulmonary TB patients of reproductive age show significantly reduced serum estradiol and progesterone concentrations relative to age-matched healthy females [143]. In post-menopausal females, mean estrogen concentrations in TB patients were found to be significantly higher than in age-matched women without TB [144]. Compared to healthy male controls, male patients with active TB (AT) exhibit significantly reduced dehydroepiandrosterone (DHEA) and testosterone titers, with moderately higher levels of estradiol, cortisol, prolactin, and thyroid hormones [145]. However, the existence of serum testosterone in three different forms (free, globulin-bound, and albumin-bound), as well as its conversion to estrogens, further complicates the assessment of its effects on TB. Although the major male androgen is testosterone, the effects of other important androgens such as DHT and androstenedione on immune responses are less well characterized.

These sex steroids are known to influence immune responses positively or negatively, most prominently in the reproductive years of females. They can modulate the proliferation and immune function of monocytes, macrophages, neutrophils, dendritic cells (DCs), natural killer (NK) cells, and B and T cells. The human ERα estrogen receptor (ER) is strongly expressed on immune cells within the TB granuloma, including DCs, monocytes, macrophages, NK cells, and T cells, whereas ERβ appears mostly on B cells and subset of T cells [68]. Androgens mainly function by binding to their cognate androgen receptor (AR). In addition to the AR, testosterone also cross-binds with the progesterone and ERs, although with a lower affinity, whereas DHT binds more specifically to the AR. Genes encoding many immunostimulatory proteins (e.g., IFN-γ, TLR7) have hormone-response elements (HREs) in their promoters, which allow bound sex hormone receptors with nuclear localization sequences to act as transcription factors (TFs), leading to altered gene expression [41]. In human studies, estrogen has been deemed to have an immunoenhancing effect on the immune system, whereas testosterone and progesterone are considered to be immunosuppressive [146].

Effect on adaptive immune responses

Adaptive immunity to TB is apparent 2–6 weeks after infection, and T cells play a pivotal role in providing anti-TB immunity [42]. Cells involved in type 1 T helper (Th1) cell responses against Mtb are well characterized and are known to secrete IFN-γ, TNF-α, and IL-2. ERα-deficient mice have decreased IFN-γ-secreting cells in lymph nodes, suggesting that ERα-mediated signaling is required for Th1 cell responsiveness [66]. 17β-Estradiol can stimulate both pro- and anti-inflammatory cytokine synthesis by $CD4^+$ T cells depending on the hormone dose, as revealed by examining the effect of estrogens on cytokine secretion by human T cell clones [67]. High estradiol concentrations promote type 2 T helper (Th2) cell-biased immunity (during pregnancy or follicular phase of the menstrual cycle), whereas basal/low concentrations trigger Th1 polarization, macrophage activation, and stimulation of proinflammatory cytokines such as IFN-γ and TNF-α [41,43,68]. Because Th1 responses are key for Mtb containment, basal estradiol concentrations in the non-pregnant female might be a mechanism by which females are better protected against Mtb disease, although this remains conjectural. A recent study in which Mtb-infected human bronchial epithelial cells were treated with exogenous estradiol *in vitro* revealed that estrogen inhibited intracellular Mtb proliferation, as examined by release of LDH [69]. Conversely, as a host susceptibility factor, testosterone can promote the growth of both Mtb and NTM in murine models [26,32,33]. High testosterone concentrations have an immunosuppressive effect by inhibiting B and T cell proliferation and maturation, impairing macrophage activation, reducing IFN-γ, TNF, iNOS, NO, and **Toll-like receptor (TLR)** 4 concentrations, and by elevating IL-4 levels [68,70,71]. Moreover, testosterone signaling via the AR on $CD4^+$ T lymphocytes leads to increased IL-10 production [72]. Addition of IL-10 blocks phagosome maturation in Mtb-infected human macrophages [73]. IL-10 is also known to suppress NF-κB activation, Th1 cytokine production, ROS and nitric oxide (NO) concentrations in murine macrophages [73–75]. Thus, we speculate that elevated IL-10 in males might play a causal role in late-stage TB disease progression. Although estradiol has been shown to inhibit IL-10 production in rat spinal cord, higher blood concentrations (as seen during pregnancy) promote IL-10 synthesis [68,76,77]. In addition, the proinflammatory cytokine IL-23 shows sex-dependent variation, and higher amounts are present in female lungs during TB than in male mice [29]. Because IL-23 is involved in long-term containment of Mtb and lymphoid follicle formation [78], the reduced concentrations of this cytokine in male lungs might also play a causal role in the reported sex bias, although this remains to be tested. Notably, AR signaling is known to reduce IL-17A and IL-23R production from type 17 T helper (Th17) cells [79], whereas estrogen increases both, suggesting that Th17 helper function might be reduced in males during TB; however, this remains untested [29,79,80]. These findings provide evidence strongly suggesting that adaptive immune responses following Mtb infection might also be influenced by sex hormones.

Genetic determinants of sex differences in TB

Although sex hormones are likely to play important roles in the TB sex bias, genetic factors may also contribute significantly because the chromosome complement has key effects on human immune function (Box 4 and Figure 2). The X chromosome encodes 16 immunological receptors, 15 immune response-associated proteins, >25 regulatory genes, and a large number of miRNAs and long non-coding RNA (lncRNAs) with immunomodulatory functions [81]. Among the X-encoded protein-coding genes are *TLR7*, *TLR8*, *CYBB*, *NEMO*, *CD40L*, and *FOXP3* (Table 2) [82]. By contrast, the small ~60 Mb Y chromosome contains only 77 genes plus the **sex-determining region on chromosome Y (*SRY*)** gene which governs the development of the testes (Box 4) [83]. GTEx-generated RNA-seq data revealed that, of 34 genes with sex-biased expression in the human lung, 31 mapped to the Y chromosome; however, except for *KDM5D* and *DDX3Y*, the immunostimulatory properties of other gene products are unknown [84].

Box 4. Sex chromosomes and X chromosome inactivation (XCI)

Human females are homogametic, with two copies of the X chromosome (XX), whereas males with two different sex chromosomes (XY) are heterogametic. The ~155 million bp (Mb) X chromosome encodes >800 proteins and contains approximately 600 non-coding genes, many of which regulate immune function, including pattern recognition receptors (e.g., *TLR7* and *TLR8*), cytokine receptors (e.g., *IL13RA1*, *IL13RA2*), regulatory proteins (*NEMO*, *BTK*, *CYBB*), and TFs (e.g., *FOXP3*) [81]. The acrocentric human Y chromosome harbors ~200 genes, at least 78 of which are protein-coding and include *SRY*, azoospermia factor A and B (*AZFA*, *AZFB*), lysine-specific demethylase 5D (*KDM5D*), and histone demethylase (*UTY*) [83]. The Y chromosome hosts several genes relevant to autoimmune diseases and immune responses against infections; polymorphisms in Y chromosome-encoded genes can also influence sex-dependent susceptibility to some viral infections [147].

To suppress allele dose differences in X-linked genes between sexes, one of the X chromosomes is epigenetically silenced (XCI) randomly by the *Xist* lncRNA into an inactive Barr body during early embryonic development in females [126]. Consequently, males with a single X chromosome are more likely to have X-linked disorders, whereas females with two X chromosomes have a biological advantage owing to XCI-mediated cellular mosaicism. However, 15–30% of the genes on the inactivated X chromosome may escape transcriptional silencing ('escape from XCI'), many of which have immunomodulatory potential, thus adding to sexually dimorphic traits of X-linked genes [148]. Moreover, because proximity to the X chromosome pseudoautosomal region 1 (PAR-1) near one telomere is associated with highest likelihood of escape from XCI, immune function genes such as *TLR7*, *TLR8*, and *CYBB* are predicted to have a high probability of escaping XCI [126]. Much heterogeneity is observed in the number of genes which escape XCI, their expression across different tissues within an individual, and between sexes. **Genetic imprinting** and skewed XCI may also impart immunological advantages to females [149]. The immunological impact of the X chromosome is exemplified by males with Klinefelter syndrome (XXY) who have low testosterone and gonadotrophins, but elevated estrogen concentrations, and hence exhibit female-like higher circulating IgG titers, and B and $CD4^+$ T cell counts, than XY males. Similarly, females with Turner syndrome (X0) show immunological responses similar to those of XY males [43].

Polymorphisms in X-linked genes may account for sex differences in immune function

Human genome-wide association studies (GWAS) provide strong evidence about multiple X-linked genes influencing sex-specific susceptibility to TB. Functional polymorphisms in *TLR7* and *TLR8* are linked to TB susceptibility in humans [85–87] owing to impaired TNF-α production and increased Mtb phagocytosis in monocytes, particularly in male pulmonary TB patients, in comparison to those with the wild-type (WT) allele [87]. Six different studies performed in seven different countries have found SNPs in *TLR8* that are associated with statistically significant, sex-based differences in the development of AT [85–89], some with male odds ratios as high as 4.04 [87]. The CD40 ligand (CD40L), a TNF superfamily member expressed primarily on activated T cells, promotes B cell maturation and immunoglobulin class switching [90]. A recent case report found that X-linked **hyper-IgM syndrome** caused by mutations in CD40L was associated with recurrent TB infection [91]. In addition, polymorphisms in X-linked *CD40L* in combination with *TLR8* and *IRAK1* variants increased male susceptibility to pulmonary TB in the Han Chinese population [92]. Lastly, a promoter polymorphism in the X-linked *FOXP3* gene, that encodes the master regulator of **regulatory T cell (T_{reg})** development and function, was significantly enriched in TB patients versus healthy controls, and females with this polymorphism expressed ~fivefold more *FOXP3* RNA than healthy control females [93]. Mutations in immunomodulatory X-linked alleles conferring TB susceptibility would show a greater impact among gonadal males because they harbor only one X chromosome. Thus, the significant association of these polymorphisms, particularly in pulmonary TB males, further supports the importance of genetic variability in sex-disaggregated TB data (Figure 2).

Incomplete dosage compensation because of X chromosome inactivation escape in females

Escape from **X chromosome inactivation (XCI)** (Box 4) may account for sex differences in immune responses and disease incidence. Indeed, using tumor/germline exome sequencing data obtained from 4126 human patients across 21 different tumors, a study mapping the genetic determinants in cancers that show a ≥2:1 M/F ratio found that escape of X-linked tumor-suppressor genes accounts for the male sex bias [94]. A large number of X-linked immunocompetent genes

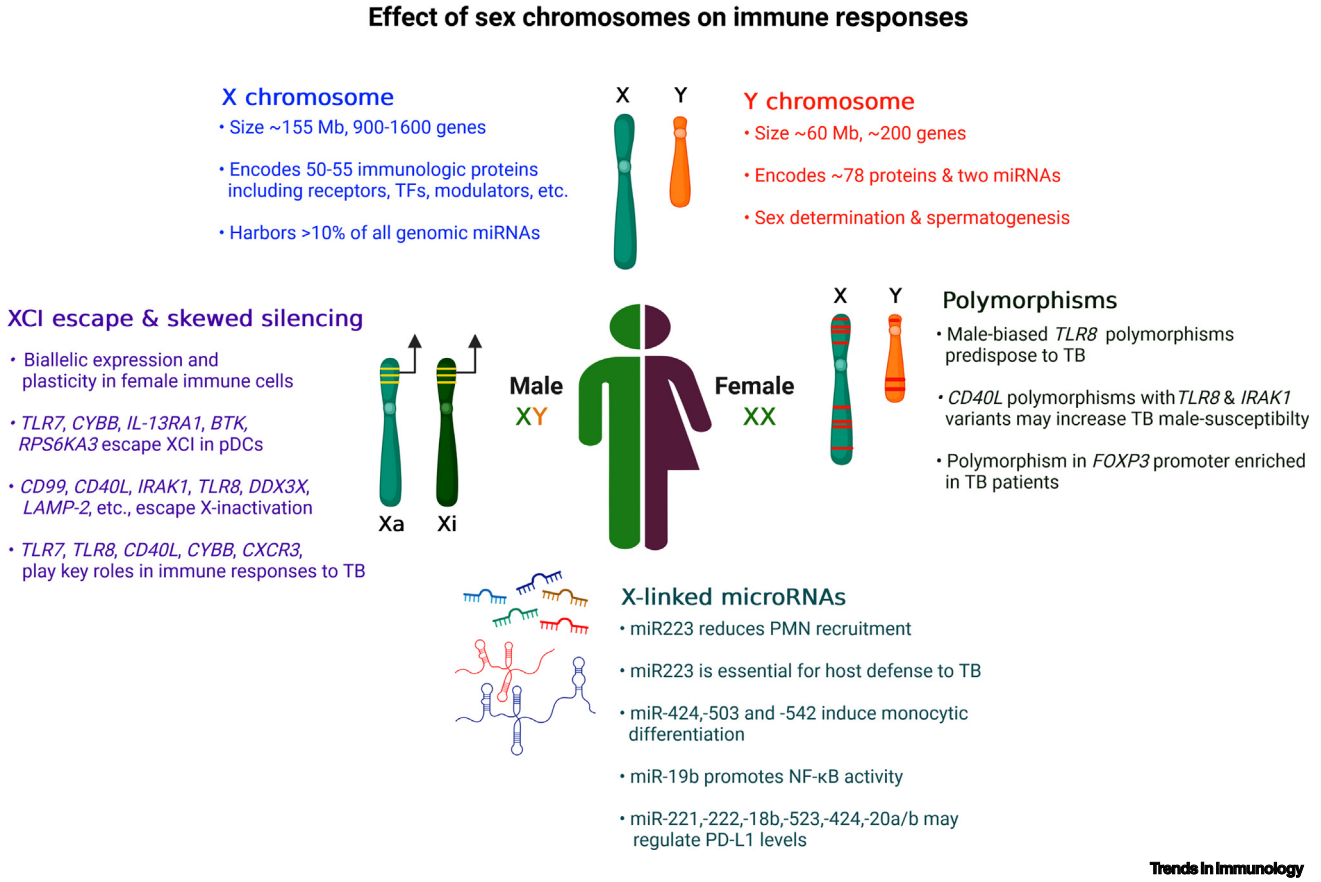

Figure 2. Various genetic events that may regulate male sex bias in anti-tuberculosis (TB) immunity. Key genetic determinants on sex chromosomes with potential roles in susceptibility to TB infection, as reported in mice or human studies. Schematic representation, images not scaled. Figure created using BioRender.com. Abbreviations: BTK, Bruton's tyrosine kinase; CD40L, CD40 ligand; CXCR3, C-X-C motif chemokine receptor 3; CYBB, cytochrome b-245 β chain; DDX3X, DEAD-box helicase 3 X-linked; FOXP3, Forkhead box P3; IL-13RA1, interleukin 13 receptor α1; IRAK1, interleukin-1 receptor-associated kinase 1; LAMP-2, lysosomal-associated membrane protein-2; Mb, million base pairs; PD-L1, programmed death ligand 1; PMN, polymorphonuclear neutrophil; RPS6KA3, ribosomal protein S6 kinase A3; TF, transcription factor; XCI, X chromosome inactivation.

including *TLR7*, *TLR8*, *CYBB*, *IL-13RA1/RA2*, *CXCR3*, *BTK*, *KDM6a*, *CD99*, *CD40L*, *IRAK1*, *DDX3X*, *LAMP-2*, and *KDM5c* are known to escape XCI (Figure 2) [95,96], thereby generating a female-specific heterogeneous population with biallelic expression. The resulting cellular mosaicism is likely to impart functional plasticity within female immune cells. Although XCI escape as a potential cause for male bias in TB has not been studied, the immunomodulatory functions of genes known to escape XCI are well established and hence might prove to be relevant in TB (Table 2). For example, *TLR7* escapes XCI in plasmacytoid dendritic cells (pDCs), monocytes, and B cells of normal women and Klinefelter syndrome males, and is known to exhibit a strong sex bias in humans and mice [97,98]. TLR7 signaling inhibits intracellular Mtb proliferation by triggering autophagy in macrophages [99]. Together with TLR9, TLR7 activation is also known to downregulate the MARCH1 ubiquitin ligase and reduce IL-10 secretion, while inducing MHC-II expression in primary BMDMs of C57BL/6 mice [100]. The induction of **class-switch recombination (CSR)** and the generation of antigen-specific antibodies rely on TLR7 engagement in B cells [101]. Indeed, type I IFN production in pDCs, induction of CSR, and the generation of antigen-specific antibodies in B cells following TLR7 signaling are all significantly higher in human females than in males [97,98,102,103]. Second, the CD40L in association with IFN-γ is

Table 2. X-linked genes or miRNAs with a potential role in anti-TB immunity[a]

Gene product	Location	Function	Human polymorphism phenotype[b]	Mutant mouse TB phenotype	Refs
TLR7[c]	Xp22	Recognition of ssRNA	↑ HCV and HIV-1	–	[150–152]
TLR8[c]	Xp22	Recognition of ssRNA	↑ TB susceptibility	–	[85,86]
CYBB[c]	Xp21	NADPH oxidase	MSMD with CGD	Susceptible	[106,108]
CD40L[c]	Xq26	Expressed mainly on activated T cells CD40–CD40L binding triggers cytokine and chemokine production	Together with *TLR8* and *IRAK1* polymorphic variants, ↑ male susceptibility to pulmonary TB	Similar to WT but impaired granuloma formation	[92,153]
IRAK1[c]	Xq28	IL-1 receptor-associated kinase 1 Regulates TLR and IL-1R-mediated signaling	Autoimmune diseases such as SLE, systemic sclerosis, and rheumatoid arthritis	–	[154]
CXorf21[c]	Xp21	TLR adaptor interacting with SLC15A4 on lysosomes	Sexual dimorphism in SLE	–	[155]
NEMO	Xq28	NF-κB essential modulator	MSMD	–	[156]
FOXP3	Xp11	Transcriptional regulator for T_{reg} cells Inhibits IFN-γ production and T cell effector function	↑ Female susceptibility to TB ↑ Viral susceptibility	–	[93,157]
IL13RA1[c] and RA2	Xq24	IL-13 receptor α1 and α2	Atopic disease	Unknown[d]	[113,114,158,159]
CXCR3[c]	Xq13	C-X-C motif chemokine receptor 3	Male bias and pleuritis in SLE	Resistant to TB	[111,160]
KDM6a[c]	Xp11	Lysine-specific demethylase 6A	Bladder cancer, B cell lymphoma	–	[161]
miR-223	Xq13	PMN recruitment	Rheumatoid arthritis	Susceptible to TB	[48,115,116,162]

[a]Abbreviations: CD40L, CD40 ligand; CGD, chronic granulomatous disease; CXorf21, chromosome X open reading frame 21; CYBB, cytochrome b-245 β chain; FOXP3, Forkhead box P3; HCV, hepatitis C virus; IL13RA1/RA2, IL-13 receptor subunits α1/α2; IRAK1, interleukin-1 receptor-associated kinase 1; miR, miRNA; MSMD, Mendelian susceptibility to mycobacterial disease; NEMO, nuclear factor κB (NF-κB) essential modulator; PMN, polymorphonuclear neutrophil; SLC15A4, solute carrier family 15 member 4; SLE, systemic lupus erythematosus; ssRNA, single-stranded RNA; TLR, Toll-like receptor; WT, wild type.
[b]Upward arrow: increase.
[c]The respective genes are known to escape from XCI.
[d]Transgenic IL-13 overexpressing C57BL/6 mice show necrosis and ↑ TB susceptibility.

known to activate vitamin D-dependent antimicrobial responses and the induction of autophagy against intracellular Mtb infection in human monocytes *in vitro* [104]. Third, the XCI-escaping gene *CYBB*, which encodes cytochrome b-245 of NADPH oxidase, is the primary component of the ROS-generating NADPH oxidase complex of phagocytes, and defects in the *CYBB* gene are known to cause **Mendelian susceptibility to mycobacterial disease (MSMD)** [105]. Mutations in *CYBB* lead to X-linked chronic granulomatous disease immunodeficiency (X-CGD). Although CGD patients have recurrent skin and soft-tissue infections, they are also known to have a higher incidence of AT than healthy controls, suggesting that the oxidative burst is perhaps crucial for host resistance to mycobacterial infections [106]. Phagocytes extracted from CGD patients show no intracellular killing and fail to limit bacterial replication upon infection with BCG under *in vitro* conditions [107]. In addition, *Cybb*$^{-/-}$ knockout mice are more susceptible to TB than are WT mice, although sex differences among these mice have not been investigated [108]. However, because *CYBB* is as an X-linked gene affecting more males than females [109,110] and is also associated with an elevated risk of TB [106], *CYBB* mutations that cause X-CGD may be genetic contributors to the sex bias in TB, even though the overall numbers of X-CGD patients are low globally. In contrast to the above examples, the XCI-escaping C-X-C motif chemokine receptor 3 (*CXCR3*) gene product is known to attenuate anti-TB immune responses in mice by altering T cell priming and granuloma composition; hence CXCR3-deficient

BALB/c mice show more resistance to Mtb infection than WT mice [111]. Lastly, the IL-13 receptor protein α1 gene (*IL13RA1*) is also known to escape XCI in human pDCs [112]. Although knockout mice for *IL13RA1* and *IL13RA2* are not available, it is known that transgenic C57BL/6 mice overexpressing IL-13 develop a more aggressive form of TB than WT mice, with large necrotizing granulomas similar to those seen in the **C3HeB/FeJ 'Kramnik' mice** [113,114]. Thus, excess signaling through IL-13 and its receptor is deleterious in some mouse models of TB, and mutations or altered expression of X-linked IL-13 receptor-encoding genes in humans may play a protective role in TB, although this certainly needs further investigation.

X-linked miRNAs

miRNAs are negative regulators of gene expression (Figure 2). The X chromosome accounts for ~10% of all genomic miRNAs, whereas the Y chromosome harbors only two [81]. To date, miRNA-223 is the most studied X-linked small non-coding RNA known to be involved in immune regulation. Compared to healthy controls with LTBI, miRNA-223 is abundantly expressed in the peripheral blood of patients with AT infection [108]. Notably, miRNA-223 is an anti-inflammatory miRNA which has been found to downregulate chemoattractants such as CXCL2 and CCL3 and thereby reduce polymorphonuclear neutrophil (PMN) recruitment, and PMN recruitment is strongly associated with lung damage in TB [48,115,116]. Indeed, *miR223*$^{-/-}$ C57BL/6 mice were found to be highly susceptible to TB infection in comparison to WT mice which are known to show resistance [116]. Thus, miRNA-223, in addition to its role in regulation of myelopoietic differentiation under homeostasis [117], appears to be crucial for immune responses and containment of TB infection; however, differential expression of miRNA-223 among males and females, and its correlation with sex differences in TB mortality, if any, remains uninvestigated. Overexpression of other X-linked miRNAs such as miRNA-424 and 503 causes cell-cycle arrest and promotes differentiation of THP-1 monocytes [81], whereas miRNA-19b potentiates NF-κB activity in both mouse and human cells [118].

Programmed death ligand 1 (PD-L1) is regulated by hypoxia-inducible factor 1α (HIF-1α) and STAT3 [119]. Activation of the **PD-1/PD-L1 immune checkpoint** pathway leads to T cell dysfunction, and anti-PD-1 antibody immunotherapy led to reactivation of TB in a 3D cell culture model of human TB, with concomitant TNF-α release and accelerated Mtb growth [120]. Notably, a large number of X-linked miRNAs can directly or indirectly regulate the expression of PD-L1. For example, miRNA-221 and 222 can potentially regulate PD-L1 expression because they regulate the transcription activator STAT3 [121], whereas miRNA-18a/b is known to regulate the TF HIF-1α in human cancer cells [122,123]. miRNAs 513, 424, 20a/b, and106a/b appear to modify PD-L1 expression by directly targeting *PD-L1* mRNA, as validated in various human cancer models [124]. Thus, sex-biased miRNA expression might influence PD-1/PD-L1 expression, which may in turn modulate TB susceptibility. However, such possibilities will require investigation in the context of TB. In addition, the immunological functions of only a few X-linked miRNAs are known in the context of anti-TB immunity, and further studies to explore their roles in TB sex differences are warranted.

Concluding remarks

Sex differences in TB are likely to be multifactorial, and elucidating the underlying mechanisms will require a thorough understanding of the social, behavioral, hormonal, and genetic factors that mediate differing immune responses in males and females. Significant gaps remain in our knowledge of how the sex chromosome complement and sex hormones influence innate and adaptive immune responses during TB, especially during the transition from latent to AT, and whether there are sex differences in response to anti-TB therapy (see Outstanding questions). Future studies in humans and in animal models to evaluate the influence of sex hormone and identify sex-linked biomarkers at different stages of TB infection will be valuable in

Outstanding questions

What are the specific hormonal mechanisms underlying female resistance to TB infection in humans? Although the sex hormones are known to have different immunological effects (Figure 1), the specific pathways modulated by the sex hormones following TB infections in males and females remain unknown.

Do X-linked genes, miRNAs, and/or genes that escape XCI account for the observed male susceptibility and female resistance to TB in humans? Although there is credible evidence that polymorphisms in the X-linked gene *TLR8* are associated with increased TB susceptibility, we still do not know whether *TLR8* polymorphisms contribute to the male bias in TB incidence. Likewise, there are other immunologically important X-linked genes and miRNAs that may contribute to the sex bias. Escape from XCI has been shown to account for the male bias of some cancers, but this has not been evaluated in the context of TB.

Is there a sex bias in the progression from LTBI to AT in humans? If so, then what are the anti-TB immune response biomarkers that differ in the sexes? Despite dozens of publications on human transcriptomic signatures in AT and LTBI, sex differences in the signatures remain unclear.

Are there sex differences in response to TB therapy, especially **host-directed therapies**, and in treatment outcomes?

Why do most human infectious diseases exhibit a male sex bias? Are there generalizable sexually divergent mechanisms that account for this male bias or are there different mechanisms for different infections? The question of whether a common set of endocrine or genetic factors regulate this broader male bias to infection disease severity and lethality remains unanswered.

Box 5. Murine models to evaluate sex differences in TB

Traditionally, gonadal male or female C57BL/6 mice or gonadectomized (Gdx) BALB/c animals – with appropriate sham controls, and with and without hormonal supplementation – have been the mainstay of modeling sex differences, and this has been shown for the TB male sex bias [26,27,29]. However, sex-based phenotypes are influenced throughout life, including embryonic development, and thus reflect a combinatorial effect of sex hormone organizational (prenatal) and activational (post-pubertal) effects, alongside genetic factors [163]. Indeed, transient testosterone surges occur twice in the developmental stage of most mammals including rodents, one in the prenatal phase to masculinize the gonad [164] and the second postnatal surge referred to as 'mini-puberty' to stimulate male behavior [165]. Use of adult Gdx animals, therefore, fails to include the impact of these developmental effects.

Recently, new models to discriminate the impact of genetics and sex steroids on disease, which also consider embryonic and pre-adulthood development, have been established. These include the four core genotype (FCG) and the XY* mouse models. The FCG model involves deletion of *Sry* from chromosome (Chr)Y (Y^{-}) and its translocation of onto Chr3, resulting in XY^{-}*Sry* males. Mating XY^{-}*Sry* gonadal males with XX females generates XY mice with ovaries (XYF) or testes (XYM), and XX mice with ovaries (XXF) or testes (XXM) [166,167]. These FCG mice can be studied in a 2 × 2 factorial design, and three different comparisons can be made: (i) comparing the phenotypic effect of gonads/sex steroids (XXF vs. XXM, and XYF vs. XYM), (ii) testing the effect of sex chromosome complement (XXM vs. XYM, and XXF vs. XYF), and (iii) the cumulative effect of 1 and 2 (XXF vs. XYM). In the C57BL/6 background, FCG mice of the same gonadal sex have comparable concentrations of circulating sex steroids [163], thus making them an ideal background for investigating the effect of sex chromosome complementation. Gdx FCG mice (with or without specific hormonal supplementation) can be employed to completely rule out the activation effects of sex hormones, although their long-term developmental effects cannot be controlled [163].

Phenotypic differences in gonadally identical XX versus XY FCG mice may have various genetic explanations: (i) the presence of the Y chromosome in XY mice, (ii) overexpression of genes that escape XCI, (iii) allelic mosaicism, or (iv) X-linked gene silencing in XX mice. In such situations, the second XY* model can be used to further map the genetic cause to the X or Y chromosome followed by identification of the allele itself and related downstream pathways [166]. In this model, the Y* chromosome harbors the usual *Sry* gene but also an aberrant pseudoautosomal region that leads to an abnormal recombination with the X chromosome during meiotic division. Mating such XY* males to XX females generates XX females, XO females, XY males, and XXY males [168]. Consequently, the effects of X chromosome complementation and escape from XCI on sexual dimorphic traits can be inferred by comparing XO versus XX females, or XY versus XXY males. Likewise, the effect of one or no Y chromosome can be inferred by testing XO versus XY mice, and XX versus XXY mice. In addition, the trisomy model yields eight different genotypes – gonadal male and female mice with the chromosomal complements XX, XY, XXY, and XYY. This model may also be employed to validate the effect of sex chromosome dosage [169].

understanding the male sex bias in TB and for the development of specific therapeutics. New tools such as the four core genotype (FCG) mouse model combined with advanced 'omic' techniques hold significant promise in this effort (Box 5). Similarly, comparative analyses on gene expression across blood and pulmonary samples from males and females with latent and advanced TB disease will be necessary to validate findings from animal models. Finally, it is important to recognize that the greater male biological susceptibility to TB is also observed in several viral, bacterial, and parasitic infections in humans including COVID-19, Ebola, syphilis, and leishmaniasis, to name a few [43,125,126]. However, it remains to be determined whether the male bias in these different infections has a common biologic basis or whether disease-specific mechanisms apply (see Outstanding questions). A better understanding of sex-based differences in these disparate infections will offer greater insight into infectious disease pathogenesis and mechanisms of immunity, and may enable the development of new and sex-specific interventions and therapies.

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Declaration of interests

No interests are declared.

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