Seminars in Immunopathology (2019) 41:225–237
https://doi.org/10.1007/s00281-018-0725-6

REVIEW

Sex differences in tuberculosis

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Received: 8 July 2018 / Accepted: 10 October 2018 / Published online: 25 October 2018

Abstract

Tuberculosis is the most prevalent bacterial infectious disease in humans and the leading cause of death from a single infectious agent, ranking above HIV/AIDS. The causative agent, *Mycobacterium tuberculosis*, is carried by an estimated two billion people globally and claims more than 1.5 million lives each year. Tuberculosis rates are significantly higher in men than in women, reflected by a male-to-female ratio for worldwide case notifications of 1.7. This phenomenon is not new and has been reported in various countries and settings over the last century. However, the reasons for the observed gender bias are not clear, potentially highly complex and discussed controversially in the literature. Both gender- (referring to sociocultural roles and behavior) and sex-related factors (referring to biological aspects) likely contribute to higher tuberculosis rates in men and will be discussed.

Keywords Tuberculosis · Sex differences · Male bias · Inflammation · Mouse models · Susceptibility

More Tb in men—true gender or mere reporting bias?

Tuberculosis (Tb) rates are significantly higher in men than in women. In 2016, 65% of the 10.4 million new tuberculosis cases were male [1]. However, the reported gender differences in Tb have been debated and questioned frequently. Annual estimates of Tb incidence rates reported by WHO rely on notification rates, inventory studies which help to quantify levels of under-reporting, and national prevalence surveys [1]. Notification data alone do not reliably reflect sex disparities in disease burden because the quality of surveillance systems differs considerably between countries and reporting is almost always incomplete. Moreover, differences in access to health care and the quality of sputum samples are thought to result in under-notification of women and to bias case reporting [2–5]. Prevalence surveys are an important tool to measure disease burden without care-seeking biases that affect case notifications. Importantly, the vast majority of national Tb prevalence surveys in high-burden countries have confirmed systematically higher burden of Tb disease among men, with ratios ranging from 1.2 in Ethiopia to 4.5 in Vietnam indicating that notification data even understate the male share of the Tb burden in some countries [1]. Importantly, the analysis of 29 prevalence surveys by Borgdorff and colleagues revealed that female cases were actually more likely to be notified than male cases in most surveys [6]. These results have been confirmed by a more recent analysis by Horton and coworkers [7]. Likewise, a former study conducted in Vietnam 2006–2007 found a M:F prevalence ratio of 5.1:1 even though female Tb cases were significantly more likely to be reported than male cases [8]. The male bias is also apparent in low-burden countries with no obvious difference in access to health care between the sexes such as Germany and the USA [9, 10]. In Germany, the annual Tb incidence was 7.2 cases per 100,000 population in 2016 [10]. Tb incidence was much higher in men compared to that in women (9.9 vs. 4.6; M:F ratio of 2.2). While the majority of Tb cases had foreign nationality (69.1%), the male bias was independent of the country of origin and also apparent in German citizens, although to a lesser extend (2.2 vs. 1.6).

Valuable information on sex differences in infection and disease progression can be obtained from studying the prevalence of latent Tb infection (LTBI) in men and women. While some investigators observed higher prevalence of LTBI in males compared to females [11, 12], no significant gender differences in LTBI were found by others [13–15]. A recent

This article is a contribution to the special issue on Sex differences in immunity – Guest Editors: Hanna Lotter and Marcus Altfeld

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study on risk factors for Tb in Germany revealed that latently infected household contacts showed a balanced gender distribution while active Tb was more prominent in men [16], suggesting an increased risk for disease progression in men. It should however be noted that there is no gold standard for LTBI diagnosis. Discordant tuberculin skin tests (TST) and IFN-gamma release assays (IGRA) have been reported frequently [17] and might explain the discrepancies between different studies in the past.

Overall, these studies argue against a mere reporting bias and raise the question about the underlying reasons that are responsible for higher Tb rates in men compared to women.

Sociocultural roles and behavior

Tb is mainly a disease of low- and middle-income countries, and it is widely believed that socioeconomic and cultural factors are responsible for the observed gender bias. Due to their different social roles, men are thought to be at greater risk to be exposed to *Mycobacterium tuberculosis* (*Mtb*) [18]. In some cultures, men spend more time outside their home and have more social contacts which can increase the risk of infection. Also, certain occupational risk factors are higher in men, as they engage in professions such as mining which is associated with an increased risk for Tb [19]. However, social contacts and occupational risk differ significantly between cultures and do not adequately explain why globally more men are notified with Tb than women. Moreover, indoor air pollution caused by the usage of solid fuels for cooking is widespread in some countries and has been recognized as an independent risk factor for Tb [20] which would mainly affect women.

In high-burden countries, smoking is usually more frequent in men, and several studies have established links between smoking and the risk of developing Tb [21–23]. Likewise, alcohol consumption is regarded a risk factor for Tb [20] and higher in men than in women [24]. While both smoking and alcohol intake may put men at greater risk of developing Tb than women, adding at least in part to the observed gender differences [21], these factors are unlikely to explain the consistent global male bias. In line with this, the 2016 national Tb prevalence survey in the Philippines revealed male sex as a significant risk factor independent of smoking [25] while the risk of Tb further increased in both male and female smokers but more prominent in males.

Despite the higher prevalence in men, Tb has severe consequences for women's health and is among the top five causes of female death in the world [26]. Some studies suggest that progression to disease and mortality are higher in females during their reproductive years [2]. Pregnancy increases the risk of Tb disease progression and might thus contribute to the increased vulnerability of women in those years of life [27, 28]. Nevertheless, although Tb has a huge impact on women's health, global mortality rates are higher in males compared to those in females in all age groups ≥ 15 years [1].

The impact of coinfections on the sex bias in Tb

In populations where *Mtb* is endemic, numerous parasitic, bacterial, or viral infections are coendemic and many individuals likely experience combined infections. Like in Tb, one sex is often more severely affected by certain infections than the other [29, 30]. Thus, coinfections may increase susceptibility to *Mtb* predominantly in one sex and thereby cause differences in Tb rates between men and women. One of the strongest risk factors predisposing for *Mtb* infection and progression to active Tb is HIV coinfection [31]. HIV disproportionately affects women [32]. In South Africa, one of the 30 high Tb/HIV burden countries, 12.6% of the population is HIV positive with a M:F ratio of 1:3.4 in 2017 [33]. Despite the much higher prevalence of HIV among females, Tb prevalence and mortality in men remain higher [1]. Nevertheless, HIV significantly contributes to the high susceptibility to Tb of women in their reproductive years as Tb rates are up to ten times higher in pregnant HIV-positive compared to those in HIV-negative women. Moreover, those coinfected with HIV/Tb face far higher risks of maternal mortality than those without HIV infection [34].

Other viral infections have been discussed to have an impact on Tb disease progression. In 2009, the WHO reported a substantial number of deaths in patients with chronic respiratory conditions and raised concerns about the possible impact of influenza on patients with active Tb [35]. An association between Tb and influenza virus infection and increased mortality has been observed before, with first reports dating back to the devastating Spanish Flu in 1918/1919 [36–39]. More recently, human cytomegalovirus (HCMV) has been suggested to promote the progression from latent to active Tb [40]. Like HIV, both influenza and HCMV cause higher morbidity in females particularly during their reproductive years [40, 41]. Nevertheless, these viral infections could, independent from disease severity they cause, have detrimental consequences for *Mtb* infection in both sexes and may modulate crucial immune pathways differently in males and females. Immune responses in Tb and potential sex differences will be discussed in more detail below.

Parasitic infections including protozoan parasites and helminths are among the most prevalent infections in humans in developing countries and exhibit broad geographic overlap with areas of high Tb burden [42, 43]. In general, the prevalence and intensity of parasitic infections are higher in males than in females [44, 45]. However, with respect to helminths, this can vary between different species and for some, women have been

reported to be more affected [46, 47], or there was no sex bias at all [48]. Untreated helminth infections are often chronic but rarely fatal; however, their immunomodulatory effects can have a great impact on other diseases as well as on vaccination efficacies. Helminths are potent inducers of Th2-type responses and regulatory T cells (Tregs) which are known to interfere with Th1-type immune responses [49, 50]. As such, helminths have the potential to affect responses to BCG vaccination as well as to Tb-specific immunity [43, 51]. For instance, in vitro T cell proliferation in response to BCG was attenuated in helminth-infected patients but recovered if Tregs were removed from the test cultures [52]. Likewise, cellular immune responses to *Mtb* purified protein derivative (PPD) were reduced in persons with concurrent helminth infections and could be improved by anthelmintic treatment [53]. Moreover, BCG vaccination significantly improved PPD-specific immune responses in the dewormed group only [53, 54]. Studies on *Mtb*-infected subjects have revealed diminished Th1 and Th17 responses to mycobacterial antigen in those individuals with concurrent chronic helminth infections [55]. In another study, Tb patients with helminth coinfections showed depressed anti-*Mtb* immunity and presented with more severe radiological pulmonary disease [56]. In good agreement, experimental animal studies provide evidence of impaired resistance to *Mtb* infection or reduced efficacy of BCG vaccination during helminth coinfections [57–59]. Taking together, helminth infections may interfere with immune responses to BCG and *Mtb*, but any potential differences between the sexes remain to be elucidated.

Malaria, a vector-borne disease caused by the protozoan parasite *Plasmodium*, is highly prevalent in populations where *Mtb* burden is high. Severe forms mainly occur in children or visitors of endemic areas while continuous or repeated exposure to the parasite leads to some degree of immunological protection [60]. However, sterile immunity is never achieved and persistent infections are very common among people living in malaria endemic areas. Data on sexual dimorphism in malaria are inconclusive. Recent studies found a sex bias towards men in human clinical and asymptomatic malaria [61–63]. This is supported by some experimental data which show a male bias in murine malaria models [64–67]. Both symptomatic and asymptomatic malarial infections can cause immune modulation [68], and malaria is associated with an increased susceptibility to secondary infections such as *Salmonella*, Herpes virus, and Epstein-Barr virus [68–76]. Only few studies have addressed the impact of concurrent malaria infection on the outcome of Tb. A study carried out in Guinea-Bissau found that malaria prevention can reduce mortality in severely ill Tb patients [77]. Rodent models of malaria-Tb coinfection revealed impaired control of experimental mycobacterial infection during coinfection with *Plasmodium* [78–82]. While these studies emphasize the negative impact of malaria on the course of mycobacterial infections, sex differences have not been addressed.

In conclusion, coinfections likely modulate susceptibility to *Mtb* infection in both sexes but whether a certain coinfection contributes to or masks the gender bias in Tb is very difficult to assess.

Sex differences in respiratory tract infections

Sex bias among pulmonary diseases is not restricted to Tb. A growing number of studies have described sex differences in the incidence and severity of respiratory tract infections (RTI) [83–85]. Falagas and colleagues have extracted data from 84 relevant studies that provided information regarding sex differences in the incidence and severity of common RTIs. They found that males develop RTIs more frequently than females, and usually, the course of disease is more severe, leading to higher mortality in males, especially in community-acquired pneumonia. Likewise, males of all age groups were more frequently notified with Legionnaires' disease than were females in Europe and the USA [86, 87]. While behavioral risk factors such as those discussed for Tb might also be involved, experimental studies suggest a significant role for biological factors in sexual dimorphisms in RTIs [88–90]. Although testosterone in general is thought to act immunosuppressive, males more likely seem to be affected by infection-induced inflammation compared to females in respiratory diseases [91]. Animal models with various respiratory pathogens demonstrated that the severity of symptoms usually correlated with a strong innate immune response at the early phase of infection. For example, in a model of invasive pneumonia, male mice showed significantly higher mortality upon infection with *S. pneumoniae* compared with female mice [88]. This was associated with much higher levels of cytokines and chemokines indicative of excessive Th1-type responses in males. In good agreement, animal models studying lung injury following trauma or LPS instillation have consistently found markedly improved recovery to be associated with high estrogen levels [92, 93]. Estrogen mainly acts via two intracellular receptors designated as ER-α and ER-β. Rat lung tissue was found to be particularly rich in ER-β, and estrogen treatment induced attenuation of lung injury via ER-β activation in male rats [92]. In conclusion, experimental data suggest that estrogen suppresses lung inflammatory responses and thus that the hormonal milieu in the lung greatly impacts on the outcome after infectious or non-infectious insults.

The role of biological sex in Tb

Many studies in humans and experimental animals have established clear links between sex-specific factors and the differential susceptibility of males and females to a number of infectious diseases [29, 30]. Sex-specific determinants of

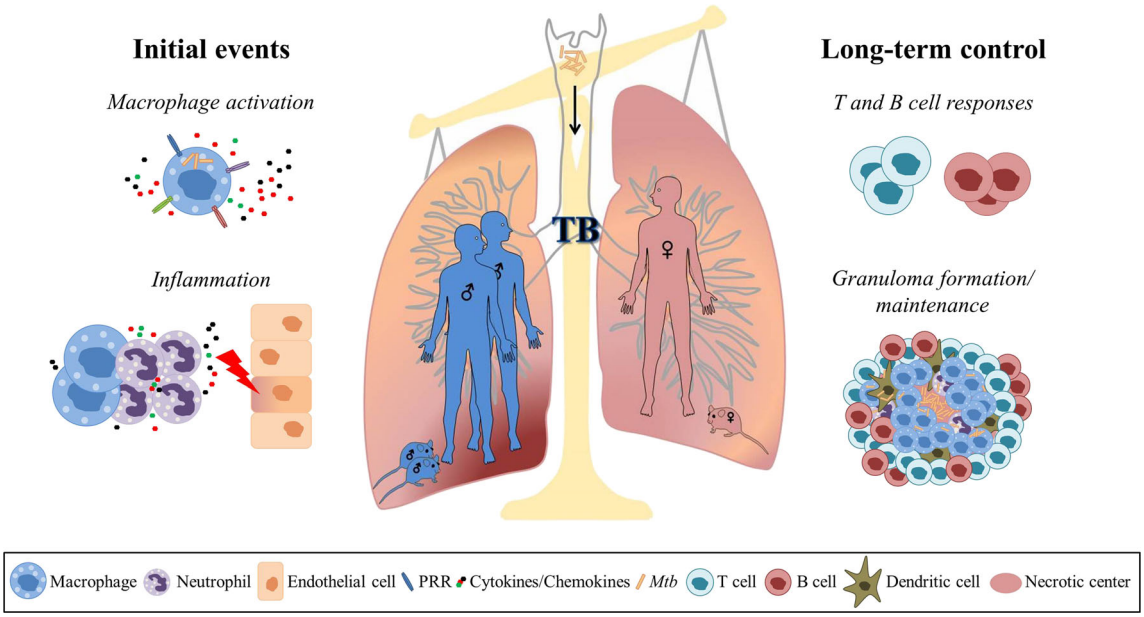

Fig. 1 Chromosomal sex and hormones might modulate the immune response to *Mtb* and thereby influence disease outcome. Macrophages are the first cells that encounter *Mtb* and initiate a local inflammatory reaction which may differ between the sexes. Consequently, the ensuing adaptive immune response which is required for long-term containment of infection may develop very differently in males and females. While studies on sex differences in humans are complicated by the influence of various confounding factors, mouse models that reflect the epidemiological observations allow for the analysis of the molecular basis of sex dependency in Tb as they are largely free of confounding variables related to behavior and exposure and particularly adequate to perform hormone or genetic manipulation

immunity include effects of sex steroid hormones as well as sex chromosome–encoded genes and microRNA (miRNA). In order to improve our understanding about the role of biological sex in Tb, mouse models that recapitulate the male bias observed in human disease can be used to dissect the molecular basis of sex dependency in Tb (Fig. 1).

Sex differences in mouse models of mycobacterial infections

Despite the well-known gender bias in human pulmonary Tb, a majority of experimental animal studies either do not separate and analyze data by sex or do not report the sex of their subjects at all. Therefore, there is clearly a lack of information on the role of biological sex in Tb. Already 30 years ago, animal studies revealed an increased susceptibility of male mice to infections with non-tuberculous mycobacteria [94–97]. Increased susceptibility was reflected by more severe gross lesions and increased numbers of mycobacteria in the organs analyzed. The studies by Yamamoto on *M. intracellulare* and *M. marinum* indicated that the male bias was mainly due to sex-specific differences in innate immunity. Peritoneal macrophages from males promoted the growth of mycobacteria much better than those from females, suggesting inherent differences in antimicrobial activities between the sexes [97]. Moreover, increased male susceptibility was also observed in T cell–depleted animals [96]. A protective role of estrogen in mycobacterial infection was suggested by Tsuyuguchi and colleagues who found impaired control of *M. avium* in ovariectomized females compared to intact females and ovariectomized females treated with 17β-estradiol [98].

More recent studies investigated sex differences in susceptibility to aerosol infection with *Mtb* in BALB/c and C57BL/6 (B6) mice. Both strains are regarded resistant to infection with reference *Mtb* strains such as H37Rv because they effectively control the growth of *Mtb* in the lungs as soon as adaptive immunity kicks in (around 20 days post infection). Consequently, disease progression is slow and resistant mice survive low-dose infection significantly longer than susceptible strains such as DBA/2 [99]. B6 and BALB/c mice are frequently used to dissect protective immune responses in Tb. A recent study by Bini and colleagues revealed that male BALB/c mice are more susceptible to *Mtb* infection than females [100]. Males showed significantly higher mortality and bacilli burdens during late disease than females and castrated males. Moreover, females and castrated males exhibited significantly higher inflammation in the lung, suggesting an immunosuppressive and detrimental role for testosterone in Tb pathogenesis. Studies in our lab revealed an increased male susceptibility towards *Mtb* in B6 mice [101] indicating that sex differences in Tb are independent of the genetic background of the host. Disease progression was accelerated in B6 males, and consequently, the majority of males succumbed to *Mtb* infection whereas 90% of females survived the observation period without overt clinical symptoms. *Mtb* numbers in male lungs were significantly elevated but controlled at a

steady level, suggesting that males did not succumb to unrestricted bacterial replication. Instead, and in contrast to what has been reported for BALB/c mice, we measured significantly increased levels of pro-inflammatory cytokines and numerous chemokines in male lungs indicative of an excessive inflammatory response compared to females. The inflammatory environment in the lung is central to the success or failure of protective immunity in Tb [102]. In fact, susceptibility to Tb can result from either inadequate or excessive acute inflammation. Too little inflammation may result in unrestricted bacterial replication while too much inflammation will cause tissue damage and immunopathology.

Sex differences in inflammatory responses following *Mtb* infection

The activation of innate immune responses is initiated by the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRR). Several classes of PRRs are involved in the recognition of *Mtb*, including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), and Nod-like receptors (NLRs) [103]. Among the TLR family, TLR2, TLR4, TLR8, and TLR9 seem to play a major role in the initiation of innate immune responses against *Mtb* [103]. The expression of PRR differs between the sexes due to the influence of sex steroid hormones, such as testosterone, estradiol, and progesterone, or location of the respective genes on the X chromosome [104]. While females have two X chromosomes and benefit from an advantageous genetic diversity due to cellular mosaicism and genes escaping X chromosome inactivation [105], males rely on the one copy inherited from their mother. Cellular mosaicism is often advantageous because it ameliorates the deleterious effects of X-linked mutations. Accordingly, a polymorphism in the TLR8 gene which is located on the X chromosome is associated with susceptibility to pulmonary Tb in men [106]. Elevated levels of TLR8 transcript were detected in active Tb patients, indicating that increased TLR8 activity is associated with Tb disease. In line with this, the expression of human TLR8 in mice resulted in significantly higher mycobacterial load in the lungs of TLR8 transgenic mice compared to those in B6 mice [107]. Not only the expression of X chromosome–encoded TLRs differs between the sexes. Male macrophages have been shown to express higher levels of TLR4 compared to those of females [108]. This increased expression was suggested to contribute to the enhanced susceptibility following LPS toxic shock, where males present with a deleterious inflammatory cytokine response [108]. In murine cytomegalovirus infection, males showed higher expression of TLR9 compared to females, which was accompanied by a lower activation of the innate immune system in female mice [109]. Hence, cytomegalovirus infection influences innate immunity differently in males and females. As mentioned above, HCMV has been suggested to promote the progression from latent to active Tb [40]. If HCMV stimulates cellular responses involved in immunity to *Mtb* differently between the sexes, this may be one scenario whereby coinfections can contribute to the sexual inequality in Tb.

An over-activated innate immune system in response to *Mtb* infection as a result of increased expression of TLR or other innate receptors in males might have considerable consequences for disease progression. It is well known that cytokines which are critically required for resistance to *Mtb* infection, such as IFNγ, IL1, or TNFα, become potentially destructive when produced unrestricted. For instance, IL1β production needs to be controlled at the inflammasome level in order to prevent detrimental innate inflammatory responses during experimental *Mtb* infection [110], and a polymorphism in the human *IL1B* promoter region associated with increased production of IL1β is associated with active Tb, severity of disease, and poor treatment outcome [111]. Recently, Sakai and colleagues elegantly demonstrated that increasing pulmonary IFNγ production exacerbated *Mtb* infection and that negative regulation of IFNγ production is required to prevent lethal immune-mediated pathology [112].

It has become increasingly clear that early events in *Mtb* infection are of major importance in dictating clinical outcome [113]. Data from non-human primate models suggest that between 3 and 6 weeks post infection, one can predict whether the animal will progress to active Tb or remain latent until 6–9 months later [114]. The numbers of initial granulomas and increasing inflammation in the first 6 weeks were associated with development of active Tb. Moreover, early greater IFNγ production was observed among macaques that would later develop active Tb than for those that developed latent infection [115]. These studies suggest that the activation status of the very first cells that get infected dictates clinical outcome as these earliest events will influence the inflammatory response, recruitment, and activation of innate and adaptive immune cells, and eventually success or failure in controlling the infection. Thus, inherent functional differences between male and female innate immune cells involved in early host-pathogen interaction likely influence the overall outcome of *Mtb* infection. As such, the antimicrobial capacity of innate immune cells is crucial in the context of infectious diseases. The phagocytic and antimicrobial activity of macrophages is higher in females than in males [116]. Superior resistance of female mice to pneumococcal pneumonia was associated with greater clearance of bacteria from the lungs due to estrogen-mediated activation of lung macrophage nitric oxide synthase 3 [89]. As discussed above, studies on non-tuberculous mycobacteria also suggested impaired antimicrobial activities in macrophages from male compared to those from female mice [97]. In line with this, we observed increased bacterial loads in male lungs as early as 12 days after *Mtb* infection [101]. Another important innate cell population involved in

the control of *Mtb* is that of invariant natural killer T (iNKT) cells. Several studies in mice and humans have shown that activated iNKT cells contribute to host resistance to *Mtb* by the production of cytokines such as IFNγ and GM-CSF and their antimycobacterial activity [117–120]. Moreover, a decrease of iNKT cells in the blood is a marker of active disease compared to latent infection or healthy controls [121]. iNKT cells are major regulators of early innate immune responses and respond to sex hormones in that estrogens enhance and testosterone decreases a pro-inflammatory immune response in these cells [122–124]. However, potential differences in their frequency or activity during an *Mtb* infection in males or females have never been addressed.

The X chromosome not only encodes for a number of genes involved in innate immunity but is also rich in miRNAs [104]. These small non-coding RNAs regulate gene expression at a posttranscriptional level and play a critical role in maintaining immunological homeostasis [104]. Sex differences in miRNA expression can be a result of their localization on the X chromosome but also of hormonal regulation as sex hormones can directly bind to the promoter elements of miRNAs [125]. For example, estrogen enhances the expression of miRNA-223 [126]. miRNA-223 is upregulated in the blood and lung of Tb patients and *Mtb*-infected mice [127]. Deletion of miRNA-223 rendered mice highly susceptible to *Mtb* infection and was associated with neutrophil-driven inflammation. In mice, neutrophil influx is associated with disease exacerbation [128]. Neutrophils are only recruited in small numbers to the site of infection in resistant mice such as B6. In contrast, the recruitment of high numbers of neutrophils is associated with tissue pathology in susceptible mice [129–131], and neutrophil depletion extends the survival of susceptible animals. Likewise, severe Tb in humans correlates best with neutrophil abundance [132, 133], and patients with active Tb display a type I IFN neutrophil-driven blood transcriptional signature [134]. In line with this, a recent report demonstrated that circulating collagenases are elevated in Tb, and identified matrix metalloproteinase 8 (MMP-8) as a novel marker of Tb compared to other respiratory infections [135]. MMP-8, also known as neutrophil collagenase, is a collagen cleaving enzyme released by neutrophils and involved in the breakdown of extracellular matrix. Importantly, MMP-8 concentrations were significantly higher in men than in women with Tb. Sex differences in neutrophil recruitment have been demonstrated in other infectious and non-infectious diseases. For example, excessive neutrophil accumulation in the lung was associated with reduced survival and pathology following *S. pneumoniae* infection or LPS challenge in males [88, 93]. Likewise, neutrophil accumulation was substantially higher in males in acute non-infectious inflammatory responses such as ischemia/reperfusion in mice or skin injury in humans [136] and shown to be a consequence of sex-specific regulation of chemokines CXCL5 and CXCL6 which control neutrophil recruitment. Taken together, sex-dependent regulation of neutrophil recruitment could have a major impact on differences in Tb disease progression and severity between females and males.

Lipid mediators play an important role in innate immunity against *Mtb* by modulating inflammatory responses during infection. Studies investigating the greater susceptibility of males to bacterial sepsis revealed that female macrophages produced significantly higher levels of the bioactive lipid PGE_2 [108]. PGE_2 has been shown to induce IL-10 production by macrophages [137–139] which has a central role in infection by limiting the immune response to pathogens and thereby ameliorating immunopathology [140]. IL-10 can impair protective immunity to Tb and impede mycobacterial clearance, but too little or no IL-10 may cause fatal host-mediated pathology [141]. Skewing immunity towards a robust pro-inflammatory state is linked to the onset of active disease [142], and severe clinical presentations of Tb are associated with strong pro-inflammatory responses [143]. This again emphasizes the importance of well-balanced pro- and anti-inflammatory immune responses during *Mtb* infection. PGE_2 not only stimulates the production of IL-10 but also protects from necrotic and induces apoptotic cell death of infected macrophages [144]. Apoptosis is an innate defense mechanism that facilitates the elimination of *Mtb* [145, 146]. In contrast, lipoxin A4 (LXA4) induces a necrotic cell death which is advantageous for mycobacterial growth and propagation [144]. Virulent *Mtb* stimulates the production of LXA_4 and inhibits PGE_2, thereby inducing an environment that favors its growth. Balanced production of lipid mediators is required for protection against Tb and may be influenced by sex hormones as indicated above [108]. The importance of the right balance is probably best exemplified in a study by Tobin and colleagues who found that in zebrafish, susceptibility to *M. marinum* can result from either inadequate or excessive acute inflammation [147]. Modulation of the leukotriene A4 hydrolase (LTA4H) locus, which controls the balance of pro- and anti-inflammatory eicosanoids, resulted in either suppressed or excessive production of TNFα. Remarkably, both phenotypes succumbed to *M. marinum* infection, demonstrating that both failed immunity and damaging hyperimmunity can lead to fatal outcome.

In summary, local inflammation critically contributes to the outcome of the granulomatous response in Tb, and a balanced production of inflammatory mediators is required for protection. Depending on host immunoregulatory factors, the antimycobacterial response could diminish or aggravate. Several genes involved in the innate immune response are located on the X chromosome. Moreover, multiple pathways can be influenced by sex hormones and skew the innate immune response into one direction or the other. To what extend sex differences in innate immunity contribute to the male bias

in Tb remains insufficiently understood and requires much more attention by researchers in the future.

Sex differences in adaptive immune responses to Tb

$CD4^+$ T cells are the primary mediators of protective immunity against *Mtb* [148]. What we had learnt from gene-deficient mice was soon confirmed in humans when realizing that the HIV-mediated loss of $CD4^+$ T cell rendered patients highly susceptible to Tb [149]. Despite the fact that immunity to Tb critically depends on an effective $CD4^+$ T cell response, nothing is known about potential differences in T cell responses between men and women with active or latent Tb. We can only speculate based on what is known about sexual disparities in T cell responses in other infectious diseases and the data obtained from the few Tb animal studies published to date.

T cell immunity is initiated in the lung-draining mediastinal lymph nodes [150] and requires dissemination of *Mtb* from the lung to the site of T cell priming [151]. Dissemination of *Mtb* is under host control and has been shown to be faster in resistant mouse strains compared to that in susceptible mouse strains [151] indicating that it is an important prerequisite for the initiation of an appropriate T cell response. Primed T cells are recruited to the site of infection in the lung where they activate antimicrobial effector functions of infected macrophages by the production of pro-inflammatory cytokines such as IFNγ and TNFα [152]. In resistant mice, this results in control of mycobacterial replication after approximately 3 weeks. Experimental studies revealed that $CD4^+$ T cells from females exhibit higher Th1 (i.e., IFN-γ) responses than those from males [104]. Our data obtained from *Mtb* infection of B6 mice do not suggest an impaired adaptive immune response in males at the early stage of infection. Although *Mtb* numbers were significantly elevated in male compared to those in female lungs, they were controlled on a constant level as soon as adaptive immunity kicked in [101]. These data rather suggest that innate immunity in males is less efficient than in females in controlling early mycobacterial replication in line with the finding from earlier studies showing that peritoneal macrophages from males promoted the growth of *M. intracellulare* much better than those from females [97]. Moreover, bacterial numbers in lung-draining lymph nodes did not differ between males and females [101], demonstrating that antigen was readily available for T cell priming in both sexes.

Continuous recruitment of lymphocytes to the site of infection leads to the formation of organized structures, granulomatous lesions in mice or granulomas in humans, which are necessary components of a successful immune response to Tb [152]. Although granulomatous lesions in mice differ from those in humans, they share several characteristics such as the formation of prominent lymphoid aggregates [153]. $CXCR5^+$ T cells exhibiting characteristics of T follicular helper cells colocalize with B cells in compact B cell follicles and are associated with immune control in mice [154]. Likewise, the development of lymphoid aggregates is associated with LTBI whereas the absence or disorganization of such aggregates is associated with uncontrolled disease in active Tb patients [155]. Consequently, it was suggested that lymphoid aggregates are a useful correlate of protection as their formation reflects proper T cell localization in the lung tissue which is a prerequisite for effective and long-lasting immune control during *Mtb* infection [154]. Our observations on *Mtb*-infected B6 mice point to an impaired formation and/or maintenance of such lymphoid structures in the male lung. Histopathological examination 5 months after *Mtb* infection revealed striking differences in the quality of the granulomatous lesions between the sexes. Female lesions featured organized lymphoid aggregates which appeared much smaller in size in males, suggesting major differences in immune cell recruitment and spatial organization [101]. Defective T cell localization could be responsible for accelerated disease progression and premature death of males compared to females because this would result in loss of control over bacterial replication [154, 156]. Sex can influence adaptive immunity to *Mtb* by defining the immunological environment in which T cell priming and granuloma formation take place. Moreover, sex hormones can affect lymphocyte function directly by binding to their specific receptors [116]. It is plausible that T cell responses following *Mtb* infection differ between the sexes; however, this does not inevitably translate into impaired immunity and increased susceptibility in males. B6 mice are known to generate a Th1 response of much higher magnitude than BALB/c mice. Nevertheless, both strains are comparable in resistance to *Mtb* infection [99]. On the other hand, despite the superior resistance of BALB/c mice compared with that of DBA/2 mice, both strains generated similar numbers of IFNγ-producing T cells [99]. This suggests that superior resistance to Tb is not necessarily based on a superior ability to generate a Th1 immune response and that in B6 mice the T cell response to low-dose *Mtb* is more robust than necessary.

In conclusion, whether T cell responses to *Mtb* differ between males and females and whether this has consequences for control of infection are not known and difficult to assess without experimental data. The ability to surgically or chemically manipulate sex hormone levels in mice provides unique opportunities to pinpoint the potential involvement of sex hormones in these processes and should be utilized in the future (Fig. 1).

Conclusion—consequences of sex differences for disease management

Tb treatment is still a major challenge. Current research strategies targeting host factors, rather than pathogen components directly, aim at the development of novel treatment

approaches termed host-directed therapies (HDT). Such approaches could augment protective immunity or minimize destructive inflammatory responses, to enhance disease resolution, improve treatment outcomes, and reduce duration of Tb therapy. What makes such approaches particularly challenging in Tb is that the disease is so heterogeneous. As discussed above, people can fall ill due to an inadequate or excessive immune response. Hence, if HDT aims at the modulation of certain immune mechanisms, we need to know the inflammatory capacity of an individual; otherwise, treatment might have detrimental consequences.

The inflammatory phenotype of an individual can be influenced by genetic background, environmental factors, age, and obviously sex. Thus, males and females may present with different clinical phenotypes and biomarkers despite suffering from the same disease. If males and females differ in their inflammatory properties, these differences need to be considered when designing and applying new (and old) therapeutic or prophylactic interventions in fighting Tb. Many approaches such as the use of corticoids or non-steroidal anti-inflammatory drugs target inflammatory processes in order to reduce tissue damage but also to facilitate the penetration of lesions by standard anti-Tb drugs to better reach the site of infection [157]. Clinical trials of corticosteroid treatment combined with standard antibiotic regimens were able to reduce Tb mortality and both pulmonary and extrapulmonary lesions [157] without detrimental side effects or relapses during follow-up. However, the response to treatment with dexamethasone in Tb meningitis has been shown to be genotype-dependent [147]. Individuals with the high-expression LTA4H genotype which was associated with hyperinflammation due to increased TNFα production benefited most from the adjunctive anti-inflammatory therapy with dexamethasone, while it remains to be determined whether dexamethasone has detrimental effects for those patients with the low-activity genotype. This example nicely demonstrates the importance of the inflammatory profile of a patient for treatment success, and this profile can be shaped by a number of factors beyond the host genotype including sex hormonal or chromosomal factors [158].

In contrast to anti-inflammatory treatments, immune activation–based therapies aim at boosting protective immune responses in order to improve Tb treatment and to shorten the duration of therapy. For example, it was suggested to stimulate host cell PRRs to augment the capacity of phagocytes to sense and internalize *Mtb* followed by processing and presentation of its antigens to T cells [159]. Clearly, such treatments bear the risk of severe side effects such as tissue damage due to hyperactivation of the immune systems and would put those subjects at risk that are prone to hyperinflammation. As discussed above, males more likely seem to be affected by respiratory infection–induced inflammation compared to females. Therefore, the immune status and inflammatory characteristics of a patient need to be considered before using immunomodulatory treatments, and sex can clearly be an important variable in these processes.

Apart from developing new therapeutic interventions, researchers around the world try to identify biomarkers which can be used to predict outcome of infection, vaccination, or therapy [160]. Such efforts could be hampered by the fact that men and women respond differently to *Mtb* infection or therapy. A major goal and challenge is to identify those latently infected individuals that are at increased risk to progress to active disease. Neither IGRAs nor the TST have high accuracy for the prediction of active Tb, and moreover, the sensitivity of TST testing differs between the sexes [161]. New biomarkers are needed which reliably predict the risk to develop active disease independent of the sex. There have been major recent advances in the discovery of blood signatures such as RNA [162] or metabolite [163] signatures which can predict progression from LTBI to active disease. Importantly, a recent study found that age and sex affected the transcriptional response of most immune-related genes to various microbial challenges [164]. Likewise, sex-related differences in the human serum metabolome covering a variety of different pathways and processes have been reported [165]. Thus, sex stratification in biomarker research seems reasonable and may not only promote the discovery of sex-specific Tb biomarkers but also the reproducibility in subsequent validation studies. Moreover, the identification of sex-specific biomarkers will deepen our understanding for differences in Tb pathophysiology between males and females. Consequently, more effective interventions can be developed for both sexes.

In summary, identifying differences in the underlying biological pathways that differentially promote Tb disease in men and women will not only widen our understanding about the interrelationship between sex and Tb, but importantly, such knowledge will also aid rational development of tailored Tb treatment regimens according to patient's sex.

Acknowledgements We thank Jochen Behrends and Ulrich Schaible for critical discussions and helpful comments on the manuscript.

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