Immunoepidemiology of *Mycobacterium tuberculosis*

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9.1 *Mycobacterium tuberculosis* (MTB): Disease Burden and Presentation

9.1.1 MTB Epidemiology

Mycobacterium tuberculosis (MTB) is the ninth leading cause of death globally and the leading cause of death from an infectious agent. In 2016, there were 6.3 million new cases of TB worldwide with 1.3 million deaths among HIV-negative and 374,000 deaths among HIV-positive subjects, respectively. The highest incident cases were reported in Southeast Asia (45%), Africa (25%), and the Western Pacific (17%) regions. Overall, the MTB incidence rate is decreasing by about 2% per year, and mortality rate is falling by about 3% per year [1]. However, 23% of the world's population (1.7 billion people) carry latent MTB [2], and poverty, crowding, undernutrition, HIV infection, and smoking have limited full eradication efforts.

9.1.2 MTB and the Human Immune System

Genetic analysis of MTB strains revealed that the pathogen emerged around 70,000 years ago and migrated with humans out of Africa. Thus, MTB and humans have been coevolving for tens of thousands of years. MTB is an obligate human pathogen that is primarily transmitted in aerosolized particles, allowing it to spread rapidly in areas of dense human habitation [3]. After pathogen inhalation, alveolar macrophages utilize numerous receptors to recognize and phagocytize the bacteria. MTB prevents its own destruction inside the macrophage by blocking fusion with toxin-containing lysosomes. The pathogen then disturbs the phagosomal membrane, allowing bacterial products and DNA to move into the

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cytosol for growth and reproduction. As the bacteria spread to alveolar epithelium and lung parenchyma, dendritic cells and inflammatory monocytes transport MTB antigens to lymph nodes for antigen presentation and T-cell priming. Primed T cells recruit immune cells to the site of infection for the generation of granulomas that serve to contain and limit the spread of the pathogen. Immune signaling molecules maintain the integrity of these granulomas, and MTB sequestered inside granulomas is known as latent MTB. If the granulomas fail, MTB activates and can disseminate to virtually any host organ, causing the deadly, contagious form of the disease. MTB phagocytosis, T-cell priming, granuloma formation, and maintenance depend on a large network of immune cascades [4].

Once infected, individuals can eliminate the pathogen, develop latent TB, or progress to active TB [4]. Latency allows the pathogen to coexist within the host for decades. Reactivation can lead to pathogen transmission to others, with infection of an entirely new population; for instance, children, who were not born at the time of initial infection [5]. Only 5–15% of individuals progress to active TB over a lifetime, but once active, untreated TB can kill up to half of those afflicted [4]. The determinants of host response are numerous and not entirely understood; however, a number of environmental, inherited, and innate factors have been identified. This chapter explores the immunoepidemiology of MTB through the discussion of representative molecules and pathways.

9.2 Impact of Other Pathogens on the Host Response to MTB

9.2.1 Malaria, MTB, and Human Immunology

Over thousands of years of evolution, infectious diseases have shaped the human genome by selective pressure. Genetic variants that confer protection against one pathogen may influence susceptibility to another. Alleles associated with the variable expression of the innate cytokine macrophage migration inhibitory factor (MIF) illustrate this phenomenon. MIF is an upstream regulator of macrophage activation, and allelic variations in its promoter, the −794 base pair CATT microsatellite, determine *MIF* expression. The microsatellite has five to eight CATT repeats. Longer length ($CATT_6$, $CATT_7$, or $CATT_8$) results in increased gene expression compared with shorter length ($CATT_5$). Although early in vitro experiments indicated that MIF inhibited the migration of inflammatory cells, such as neutrophils, we now know that MIF actually enhances the movement and activity of inflammatory cells such that higher *MIF* expression results in increased inflammatory responses. Thus, −794 $CATT_{6–8}$ alleles are associated with high *MIF* expression and stronger inflammatory responses [6]. Geographic region is significantly correlated with variant *MIF* alleles, as sub-Saharan Africa has the highest global prevalence of the low-expression −794 $CATT_5$, *MIF* allele. Specifically, −794 $CATT_5$ *MIF* alleles were reported in 78% of a Zambian population compared to only 46% of North American Caucasians [7]. These differences in allelic frequency may be due to selective pressure against lethal malaria in sub-Saharan populations, where death ensues from complications, such as cerebral disease or severe anemia, which arise as a consequence of an excessive inflammatory response. In one study of Kenyan children, those with high expression alleles were 70% more likely to develop severe malarial anemia [8]. As may occur with other pathogens, a stronger MIF-dependent inflammatory response may cause more severe disease and lethal end-organ damage in high genotypic *MIF* expressors.

While the −794 $CATT_{6–8}$ *MIF* alleles are associated with more inflammatory, severe malaria, they may also increase susceptibility to pulmonary MTB. Studies from across the globe have observed an increased risk of pulmonary MTB in individuals with high-expression *MIF* alleles. Two meta-analyses have reviewed all the largely HIV-negative subjects in these studies, each with about 1000 cases of pulmonary MTB [9, 10]. Both analyses report that −794 $CATT_{6–8}$ alleles are associated with developing pulmonary MTB, with odds ratios (ORs) that ranged from 1.5 to 1.8. By increasing local tissue

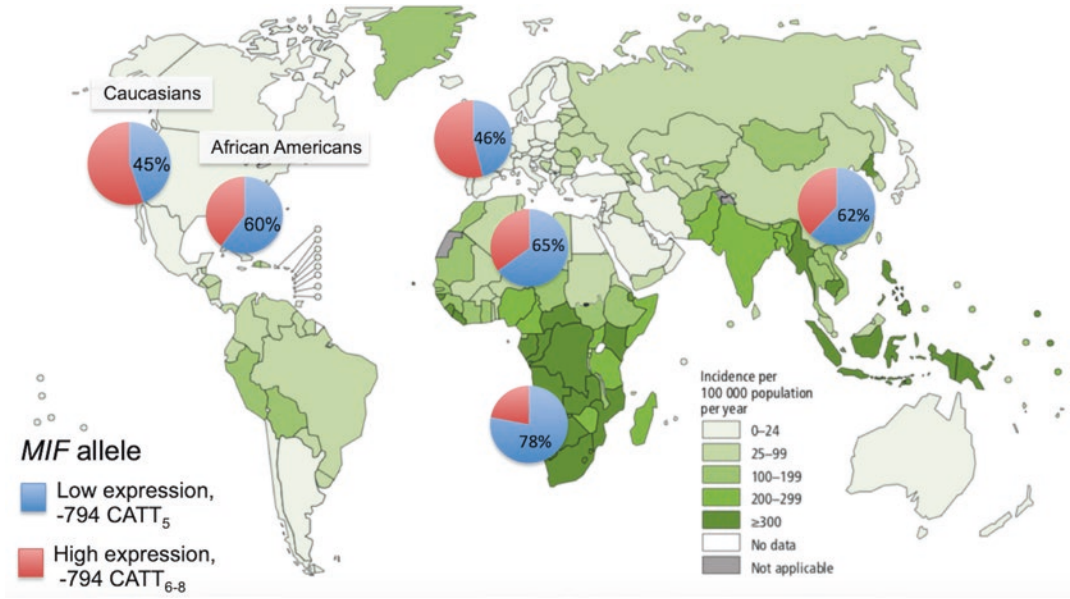

Fig. 9.1 MTB incidence rates and *MIF* allele frequencies. Superimposed MTB incidence rates, and *MIF* allele frequencies illustrate that areas with the highest MTB incidence rates (dark green) coincide with increased prevalence of low-expression *MIF* alleles

inflammation, high-expression *MIF* alleles promote alveolar lung damage, mycobacterial dissemination, and progression to active pulmonary MTB.

Notably, geographic areas with the highest incidence of MTB have the highest global prevalence of low-expression *MIF* alleles (Fig. 9.1), as has been observed with malaria. Thus, selective pressure on the immune system by regional infectious diseases likely contributes to the variability of the host response to other infections, including MTB. Although the studies discussed above suggest that the high prevalence of $CATT_5$ *MIF* alleles should protect against MTB, the opposite trend is depicted in Fig. 9.1. This is likely related to high HIV prevalence in the MTB endemic areas. Specifically, while high MIF levels worsen MTB infection in otherwise healthy individuals, those immunosuppressed by HIV may benefit from MIF's inflammatory effects for protection against MTB.

9.2.2 HIV May Alter the Role of MIF in MTB

Studies of MIF expression in HIV-infected patients suggest a distinct interaction between *MIF* alleles and MTB severity. In a South African cohort of HIV+ patients, there was a greater frequency of the low expression *MIF* allele (-794 CATT5) among MTB patients compared to HIV+ patients without MTB (OR 2.03), suggesting that the low expression allele increases the risk for MTB in HIV positive patients [11]. In a Ugandan cohort with HIV/MTB coinfection, a third of subjects with the −794 $CATT_5$ allele had MTB bacteremia, a severe form of infection, compared to only 18% of subjects with the −794 $CATT_{6–8}$ alleles. This represented an odds ratio of 2.4 even after controlling for age, sex, and severity of human immunodeficiency virus (HIV) infection [12]. Thus, high-expression *MIF* alleles may confer a survival advantage in patients with immune compromise due to HIV. While MIF's inflammatory effects may contribute to MTB progression in patients with intact immune systems, those with HIV-associated immune suppression may benefit from MIF's augmentation of host defenses to MTB.

Text Box: HIV and MTB

The emergence of the human immunodeficiency virus (HIV) dramatically altered the immunoepidemiology of MTB. In the United States, the incidence of MTB infection decreased by 6% per year between 1953 and 1985 but rose by 20% between 1985 and 1992. There was significant overlap between the patients with MTB infection and HIV, including their age, gender, and geographic location. At least half of new MTB cases were attributable to HIV [14]. In developing nations prior to the emergence of HIV, almost half the adult population harbored latent MTB and had a 1–3% risk of MTB infection per year. After 1985, there was a 6% annual rate increase in the number of MTB cases, and the hardest hit nations reported 5–8% of adults had MTB/HIV coinfection. Coinfection was associated with more severe MTB with 70% developing extrapulmonary disease compared to only 20% of HIV-negative patients. MTB associated mortality was 33% despite antibiotics versus 2% in HIV-negative people [15]. This high mortality rate was linked to synergy between the two pathogens. In vitro studies demonstrate that MTB increases HIV replication by stimulating the expression of HIV cell entry receptors and cytokines that promote viral production and decreasing protective immune signals [16]. Simultaneously, by depleting CD4 T cells, HIV cripples the immune response, facilitating granuloma degradation and MTB dissemination. HIV also inhibits immune cell migration necessary for MTB containment [13].

The rising incidence of MTB infection in settings with limited treatment management options has resulted in multidrug-resistant tuberculosis (MDR-TB). This infection is defined by mycobacterial strains resistant to at least one of the first-line antibiotics and requires longer, more expensive, and more toxic treatments. HIV infection is a major risk factor for MDR-TB with one meta-analysis reporting a 24% higher odds of developing MDR-TB in HIV-positive versus HIV-negative patients [17]. Outbreaks of MDR-TB often occurred when patients with MTB and HIV were housed together, and one hospital outbreak accounted for a quarter of MDR-TB in the United States over a 4-year period. HIV/MDR-TB coinfection had a mortality rate of 72% compared to 20% for MDR-TB alone [18]. HIV infection is associated with decreased antibiotic absorption, which further complicates treatment regimens [19]. By impacting susceptibility, antibiotic absorption, and mortality, HIV has dramatically altered the immunoepidemiology of MTB and poses a significant challenge for public health systems across the globe.

Antiretroviral therapy for the treatment of HIV reversed the grim trends of coinfection. For instance, in Atlanta, Georgia, the 1-year survival rate for patients with HIV/MTB coinfection improved from 58% in 1991 to 83% in 1997 largely due to therapeutic advances [20]. The World Health Organization (WHO) reports that 6.2 million people with coinfection were saved between 2005 and 2016, but more than a third of deaths in HIV-positive people are still due to MTB. Delay in HIV/MTB treatment is a top risk factor for developing MDR tuberculosis, and 490,000 cases of resistant MTB occurred in 2016 [1]. Overall, HIV has forever changed how MTB interacts with humanity, and global collaboration is critical to eradicating both infections.

The emergence of the human immunodeficiency virus (HIV) has had an unparalleled effect on the global burden of MTB (see text box). By impairing the “helper” CD4 T-cell response, HIV disrupts granuloma integrity and the host’s ability to maintain MTB latency, accelerating MTB reactivation [13]. Indeed, while the lifetime risk of MTB reactivation is ~10% in immunocompetent individuals [4], that risk increases to 10% per annum in HIV-infected individuals [13]. In sum, the interactions between the immune system and human pathogens are complex, and the selective pressures imposed by regional infectious diseases contribute to the immunoepidemiology of MTB.

9.3 Environmental Determinants of Host Response

9.3.1 Nutrition and MTB Susceptibility

Nutrient and micronutrient availability accounts for some diversity in the host immune response to MTB. One population-based study in the USA followed 14,279 patients over 20 years, and 61 subjects developed tuberculosis. Prior to MTB infection, 11% had low albumin, a marker of poor nutritional status, compared to only 0.5% with low albumin among those who did not develop MTB. Subjects with low body mass index (BMI) had 12.4-fold greater hazard for developing MTB versus those with normal BMI even after controlling for demographic, socioeconomic, and medical factors [21]. Gastric bypass surgery, which is used to treat obesity, intentionally decreases nutrient absorption. Those treated with this procedure have a tenfold greater risk than the general population of developing MTB, and the incidence of new or progressive infection in these patients is reported at 0.4–5% [22]. Despite these findings, nutrition intervention trials have had mixed results. A number of studies have examined the effects of macro- and micronutrient supplementation in subjects receiving treatment for tuberculosis, and no improvements in mortality or cure rates were identified [23]. It may be that nutritional status is more important for MTB infection prevention than eradication.

9.3.2 Vitamin D and Host Defense

The mechanisms whereby nutrients support immune function have not been fully elucidated but some supplements appear more central to host defense. For instance, in vitro, vitamin D has been shown to support macrophage-mediated killing of MTB [24]. In both European [25] and African [26] cohorts, vitamin D deficiency was associated with MTB infection. In a study of South African subjects, those with active MTB were significantly more likely to be vitamin D deficient than those with latent infection (OR 5.2). Moreover, seasonal variation in MTB incidence appeared to be causally related to varying vitamin D levels with significantly more MTB cases reported during the winter months [26]. In a study of Brazilian prisoners, those with active and latent MTB had significantly lower vitamin D levels than healthy controls even after adjusting for drug use, previous imprisonment, and black race (OR 3.71). However, vitamin D deficiency was not associated with progression from uninfected to latent MTB or from latent to active disease [27]. Thus, these authors conclude that vitamin D deficiency does not contribute to the development of MTB infection, but rather active MTB disease may disrupt vitamin D metabolism. In contrast, a Spanish study of subjects exposed to MTB reported that those who developed infection had significantly lower vitamin D levels than those who did not (20 ng/mL vs 27 ng/mL, $p = 0.028$) [28]. Similarly, a Pakistani study of patients exposed to MTB reported that those with low vitamin D levels had a fivefold greater risk for progressing to active disease as compared to those with normal levels [29]. In sum, these studies suggest that vitamin D levels play a role in the development of MTB infection and that MTB disease may disturb vitamin D metabolism. Interestingly, vitamin D supplementation as part of MTB treatment has not shown definitive improvement in mortality or cure rates [23]. The inconsistencies between observational and interventional trials may be related to varying doses and formulations of vitamin D supplementation, different baseline vitamin D levels among subjects, and variety in body composition that may impact vitamin D absorption and response [29].

Aside from vitamin D levels, genetic differences in the vitamin D receptor have been associated with the development of MTB. Immune cells express the vitamin D receptor, and infections stimulate its production. When bound by vitamin D, this receptor activates immune signaling pathways that support host defense. Genetic variations that decrease vitamin D receptor activity are linked to greater

MTB susceptibility. One meta-analysis of 65 articles reported a 70–90% increased risk of MTB in patients with vitamin D receptor polymorphisms that diminished downstream signaling, but these results varied by ethnicity [30]. In a separate meta-analysis, one variant of the vitamin D receptor increased the risk of MTB in a Chinese cohort, while a different polymorphism was protective against MTB in a European group [31]. Moreover, a double-blind randomized trial of vitamin D supplementation showed shorter time to MTB cure only in patients with specific vitamin D receptor variants [32]. Thus, inconsistencies in the efficacy of vitamin D supplementation may be partially related to the genetic variability of the receptor. Overall, differences in vitamin D availability and receptor genetics likely contribute to the diversity of immune responses to MTB, but more studies are needed to confirm these relationships.

9.4 Inherited Determinants of Host Response

9.4.1 Human Leukocyte Antigen (HLA) and MTB

Twin studies have demonstrated that the concordance of MTB disease is significantly higher for monozygotic (66%) vs. dizygotic (23%) twins, indicating that there is a strong genetic component to disease presentation, some of which is inherited [33]. Human leukocyte antigen (HLA) molecules are involved in antigen presentation for immune cell priming, and inherited variations in these proteins influence host defense. There are two classes of HLA genes that serve separate roles in stimulation of the immune system. Class I consists of six different isotypes that can be inherited (HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G), and class II has five isotypes (HLA-DM, HLA-DO, HLA-DP, HLA-DQ, HLA-DR). In each class, there is genetic diversity that influences the immune response by affecting antigen presentation and signaling functions. These changes are annotated according to a nomenclature of letters and numbers that will be used throughout this section. Numerous studies have identified associations between HLA types and MTB infection by assessing the frequency of each HLA type in subjects with and without disease [34]. The HLA antigens more highly represented in patients with MTB are considered risk factors for the development of infection, while those more frequently found in the healthy controls are protective. A few illustrative studies are shown in Table 9.1.

The immunogenic diversity provided by small genetic changes in HLA molecules is notable even within isotypes. One study of HLA-DRB1 reported that the HLA-DRB1∗04:11:01 subtype was associated with increased risk of pulmonary MTB (OR 2.23, $p = 0.0019$), while the DRB1∗04:07:01 subtype was highly protective against infection (OR 0.02, $p < 0.0001$) [39]. This was consistent with the low binding affinity of HLA-DRB1∗04:11:01 to MTB proteins, which limits the molecule's ability to present pathogen fragments for immune cell priming. In contrast, DRB1∗04:07:01 has high

Table 9.1 HLA classes described according to function and isotypes. HLA isotypes associated with MTB risk are listed along with the associated statistical significance

	HLA class I	HLA class II
Function	Displays intracellular antigens to CD8 T cells	Displays extracellular antigens to CD4 T cells
Isotypes	Six isotypes: HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G	Five isotypes: HLA-DM, HLA-DO, HLA-DP, HLA-DQ, HLA-DR
Increase risk of MTB	HLA-B51 ($p = 0.001$) [35]	HLA-DR8 ($p = 0.003$) [34] HLA-DQA1 ($p = 9.3\text{x}10^{-9}$) [36]
Decrease risk of MTB	HLA-B13 ($p \leq 0.0001$) [34] HLA-B27 ($p = 0.006$) [37] HLA-B52 ($p = 0.003$) [35]	HLA-DR3 ($p = 0.002$) [34] HLA-DR7 ($p \leq 0.0001$) [34] HLA-DQB1 ($p = 0.018$) [38]

binding affinity for MTB proteins allowing it to stimulate immune cells against the pathogen, which improves eradication. Other data suggest that various HLA types may have evolved to bind to specific MTB strains. One study reported that the *HLA-B*14:01* and *HLA-B*1402* alleles were associated with both Caucasian individuals and the Euro-American strain of MTB. In contrast, the *HLA-A*23:01* and *HLA-C*16:01* alleles, also more common in Caucasians, were protective against the Euro-American MTB but increased the risk of East Asian MTB strains [38]. Thus, it appears that HLA alleles associated with various ethnicities may have evolved to protect against regional MTB strains. Simultaneously, MTB strains may have mutated to evade the predominant HLA types of the region. HLA molecules provide a window into the complicated interactions between MTB and the human immune system and add to the immunoepidemiology of MTB.

9.5 SNP Polymorphisms and the Immune Response

9.5.1 Single-Nucleotide Polymorphisms (SNPs) Overview

Single-nucleotide polymorphisms (SNPs) are changes at single nucleotides in the genome and are the biggest source of genetic sequence variations in humans. When SNPs affect coding or promoter regions, which occurs in only a small minority of instances, they can alter the structure, function, and/or efficacy of proteins, thereby impacting immune responses. For example, thousands of SNPs within the HLA coding genes create the diversity of molecules discussed in the previous section. SNPs in other key immunoregulatory molecules have been associated with more severe MTB presentations contributing to the diversity of responses to infection.

9.5.2 Toll-like Receptors (TLRs) and MTB

Toll-like receptors (TLRs) bind microbial antigens and prime T cells to produce cytokines and microbicidal molecules. These receptors stretch across cell membranes with the outer portion binding proteins from viruses, bacteria, and fungi. Once activated by pathogens, the intracellular portion of the receptor is activated and initiates the innate immune system's defense against the infections (Fig. 9.2). TLRs were the first infection response proteins discovered, and nine types have subsequently been described (numbered 1–9). Given their centrality to immune function, it is not surprising that TLR SNPs have been associated with variations in MTB susceptibility.

A 16 study meta-analysis from 2013 reported that a SNP in TLR2 increased the risk of MTB (OR 5.82, $p = 0.02$), while a SNP in TLR6 decreased it (OR 0.61, $p = 0.04$) [40]. Stratification revealed that a SNP in TLR1 was associated with MTB in Africans (OR 2.47, $p < 0.01$) and American Hispanics (OR 2.12, $p < 0.01$), but these trends were not observed in Europeans or Asians. In contrast, a SNP in TLR2 increased MTB risk in Asians (OR 2.95, $p < 0.001$) and Europeans (OR 2.73, $p = 0.002$), but was not significantly associated with disease in Hispanic or Africans. Variations in immune responses by ethnicity could reflect differences in SNP frequencies among groups. Interactions between regional MTB strains and the human immune system over time could also be contributing. One research group explored these ethnic and regional differences by stimulating cells from Chinese, Filipino, and Caucasian subjects with TLR-binding proteins from four different MTB strains [41]. They reported that cells from Filipino subjects produce less inflammatory signaling molecules regardless of MTB strain. Additionally, one MTB strain elicited fewer immune responses in all cells regardless of ethnicity. Thus, both the host and pathogen play a central role in the immunoepidemiology of MTB, and further studies are needed to confirm these relationships.

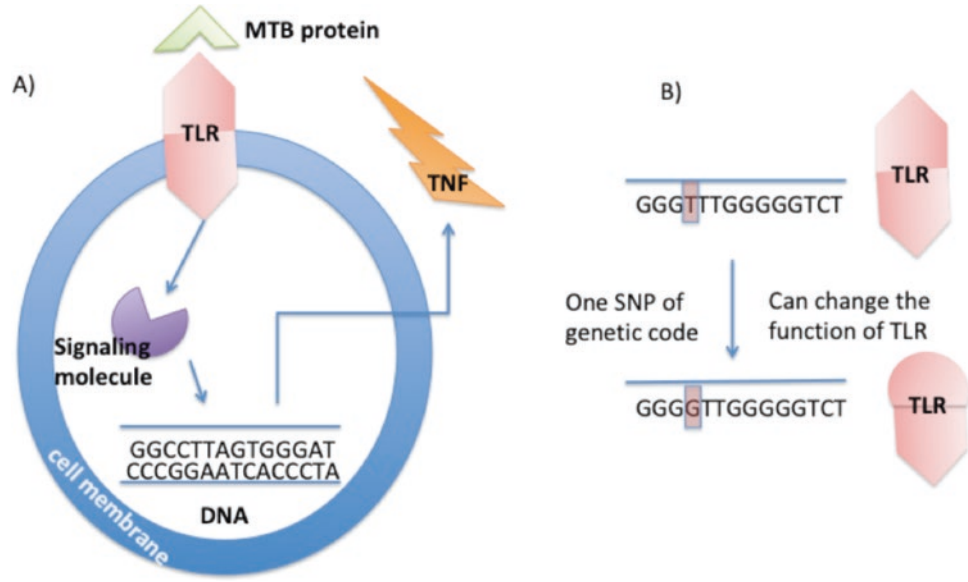

Fig. 9.2 Toll-like receptors and TB. (**a**) When TLRs bind pathogens, they change shape and stimulate signaling molecules. These activate DNA to promote the production of inflammatory cytokines like TNF for host defense. (**b**) SNPs change one nucleotide in the genetic code, and this can alter the structure and function of defense molecules

Aside from ethnicity, interesting trends in gender-related MTB susceptibility are associated with TLR SNPs. Men have a higher risk of MTB than women in large studies reporting prevalence ratios of 2.21. Reasons for this disparity likely include men having decreased access to or engagement with healthcare [42]. However, biological causes have also been discovered. In particular, because TLR8 is inherited on the sex-determining X chromosome, TLR8 SNPs are associated with gender susceptibility. One study reported that a TLR8 SNP increased with risk of MTB in women (OR = 1.41) but decreased it in men (OR = 0.72). Thus, other sex-associated signaling molecules likely interact with TLR8 variants and contribute to MTB susceptibility [43]. More gender-stratified research is needed to elucidate the biological mechanisms associated with MTB immunity.

9.5.3 Tumor Necrosis Factor (TNF) and MTB

As alluded to in the previous section, MTB binding to TLRs produces inflammatory and microbicidal pathways, which are critical for host defense. For instance, increased MTB infection rates have been documented in patients treated with blockers of the innate cytokine TNF, which is widely used for the management of autoimmune diseases. SNPs in *TNF* genes have been linked to MTB risk. A meta-analysis of 2735 cases and 3177 controls reported that two *TNF* SNPs were associated with pulmonary TB regardless of ethnicity [44]. Interestingly, separate studies linked one of these SNPs with decreased TNF expression [45] and the other with increased TNF production [46]. Thus, the associations between *TNF* genetic changes and MTB disease are likely more complicated than variations in TNF levels influencing immunity. Again, more work is needed to clarify these associations and their underlying mechanisms.

9.6 Determinants of Extrapulmonary MTB

9.6.1 IL-12-IFN-γ Pathway and Extrapulmonary MTB

Of the 10% of patients who progress to active MTB, about 20% develop extrapulmonary disease, which includes infections of the brain, bones, and liver, among other organs. The mechanisms of extrapulmonary spread are a subject of continued study. Immunosuppression, such as by HIV, cancer, and medications, is an established risk factor [47]. Extrapulmonary disease in otherwise immunocompetent hosts is still not understood, but SNPs in key genes have explained some of these cases (Tables 9.2a and 9.2b).

The most common genetic mutations associated with severe MTB occur in the IL-12Rβ1. This receptor binds the immune signaling molecule IL-12 that is produced by MTB-stimulated macrophages. When IL-12 binds to the IL-12Rβ1 receptor on T cells, IFN-γ is produced that induces free radicals to kill MTB and activates surrounding immune cells to do the same. One study of 50 children with severe MTB reported that 4% had mutations in this receptor [48]. Identifying IL-12Rβ1 mutations can have therapeutic implications since treating affected patients with IFN-γ has shown potential for curing severe MTB [49].

Given the importance of IFN-γ to host defense, a number of studies have examined IFN-γ SNPs in MTB patients. One group compared patients with extrapulmonary MTB (n = 33) and pulmonary MTB (n = 129) with healthy controls (n = 156) and reported that a *IFN-γ* SNP was more common in the MTB patients ($p < 0.0001$), although no difference in SNP frequency was observed between the pulmonary and extrapulmonary MTB cohorts. Notably, IFN-γ levels were significantly lower in the extrapulmonary MTB group as compared to the other two cohorts. This *IFN-γ* SNP occurs at an important binding site on the *IFN-γ* gene and limits the production of IFN-γ [50]. Low IFN-γ levels in

Table 9.2a SNPs affecting the IL-12-IFN-γ pathway. These have been significantly associated with severe, extrapulmonary MTB. Their function and notable lessons from their study are documented

Molecules	Function	Notable points
IL-12Rβ1 [48]	Stimulates T cells to produce IFN-γ, which is critical for MTB eradication	Patients with these mutations and severe MTB have been successfully treated with IFN-γ [49]
IFN-γ [50]	Stimulates the production of free radicals to kill MTB and activates other cells to do the same	IFN-γ SNPs and neutralizing autoantibodies increase the risk of extrapulmonary MTB. Autoantibodies against other key molecules may contribute to severe MTB
STAT1 [53]	Transmits the IFN-γ signal within the cell facilitating immune activation	Mutations in this protein highlight the IFN-γ pathway as central to preventing severe MTB

Table 9.2b SNPs affecting non-IL-12-IFN-γ pathways. SNPs in the listed molecules have been significantly associated with severe, extrapulmonary MTB. Their function and notable lessons from their study are documented

Molecules	Function	Notable points
MIF [54]	Released by infection stimulated cells; promotes inflammation	MIF associated inflammation may damage granulomas facilitating the progression to active and extrapulmonary MTB
TLR2 [55]	Cell surface receptor that binds MTB and initiates the immune response	Interactions between MTB strain and SNPs influence disease presentation
TGF-β1 [54]	Produced when TLRs bind pathogens and amplifies the immune response	The degree of infection associated inflammation may vary by SNP
P2X7 [47]	Purine binding receptor on macrophages that promotes mycobacterial killing when activated	Studying the cells from subjects with these SNPs can help identify the mechanisms whereby the mutations increase infection risk

patients with this SNP were observed in a study comparing stimulated cells with and without the SNP [51]. Another study reported IFN-γ neutralizing autoantibodies in one patient with disseminated MTB, further confirming the importance of IFN-γ in host defense [52]. Separately, SNPs in molecules downstream of IFN-γ have also been associated with disseminated MTB. Specifically, IFN-γ stimulates immune cells by activating the signal transduction and activator of transcription 1 (STAT1) protein. Mutations in this protein have been discovered in patients with severe MTB and other disseminated infections highlighting the importance of this pathway in infection control [53].

9.6.2 Other SNPs Associated with Severe MTB

Analyses of patients with MTB of the spine ($n = 110$) [54] and meninges ($n = 187$) [55] revealed associations with SNPs in two proteins already discussed in this chapter, MIF and TLR2. The high-expression *MIF* allele increased the odds of spinal MTB by 47% ($p < 0.01$), and a *TLR2* SNP increased the odds of MTB meningitis by 51% ($p = 0.006$). Notably, the TLR2 SNP association was only observed in patients with a particular MTB strain. Thus, the interaction between various MTB strains and immune system SNPs is an important contributor to extrapulmonary disease.

Activation of TLRs stimulates the production of the inflammatory cytokine transforming growth factor (TGF)-β1. The spinal tuberculosis study reported higher rates of two TGF-β1 SNPs in cases vs controls with odds ratios of 2.3 ($p \leq 0.04$). These alleles also were associated with higher inflammatory markers suggesting that genetic changes can worsen inflammation. Separately, a meta-analysis of 18 studies examined risk factors for extrapulmonary MTB and reported that a SNP in the macrophage purinergic receptor protein P2X7 was the most strongly associated with severe MTB (OR 2.28). This receptor is present on macrophages and promotes mycobacterial killing when activated. P2X7 SNP is a loss-of-function mutation, thereby explaining how the SNP increases risk for MTB spread [47]. Interestingly, this meta-analysis also combined the data from four studies examining the aforementioned *IFN-γ* SNP and did not find an association between the SNP and extrapulmonary disease (OR 1.03). Specifically, a Columbian study reported that the rate of extrapulmonary infection was higher without the *IFN-γ* SNP, while an Egyptian study reported the opposite [47]. Moreover, a Brazilian [50] and a Tunisian study reported no significant association between extrapulmonary disease and the *IFN-γ* SNP [47]. Of note, none of these studies controlled for race or MTB strain, although the Tunisian study did report a "predominant" MTB strain in the population. These analyses also were limited by small sample sizes (ranging from 30 to 50) with the largest cohort ($n = 84$) reporting the only positive association between the *IFN-γ* SNP and extrapulmonary disease. This group of studies demonstrates the challenges of identifying significant SNP-infection associations. Future studies should attempt to stratify data by race, gender, and MTB strain. Sample sizes may be enlarged by collaborative efforts among MTB treatment centers.

9.7 Conclusions

The clinical presentation of MTB is affected by a variety of host immune factors.

MTB has a large spectrum of presentation from subclinical microbe eradication to generalized dissemination, and phenotype is determined by host–pathogen interaction. A number of factors contribute to the diversity of presentations including selective pressure on immunity by other pathogens, environmental influences, inherited factors, pathogen strain, and inborn variations of

the immune system. Given the complex, multifaceted interactions, it has been difficult to develop a complete picture of the immunoepidemiology of MTB. Until now, seminal discoveries have been piecemeal and highlight the importance of generating large, clinically well-characterized cohorts that can be stratified according to influential demographics. Armed with this knowledge, we expect more comprehensive studies will produce prognostic and therapeutic response algorithms to improve preventative and curative treatments. Lessons learned also inform the general study of host–pathogen interactions and the mechanisms underlying diverse disease presentations.

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