ANNUAL REVIEWS

Annual Review of Pathology: Mechanisms of Disease

Perspectives and Advances in the Understanding of Tuberculosis

Rachel L. Kinsella,* Dennis X. Zhu,* Gregory A. Harrison,* Anne E. Mayer Bridwell,* Jerome Prusa,* Sthefany M. Chavez,* and Christina L. Stallings

Department of Molecular Microbiology, Washington University School of Medicine, Saint Louis, Missouri 63110, USA; email: stallings@wustl.edu

Annu. Rev. Pathol. Mech. Dis. 2021. 16:377–408

The *Annual Review of Pathology: Mechanisms of Disease* is online at pathol.annualreviews.org

https://doi.org/10.1146/annurev-pathol-042120-032916

*These authors contributed equally to this article

Keywords

mycobacteria, pathogenesis, infection, host response, virulence

Abstract

Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis (TB), remains a leading cause of death due to infection in humans. To more effectively combat this pandemic, many aspects of TB control must be developed, including better point of care diagnostics, shorter and safer drug regimens, and a protective vaccine. To address all these areas of need, better understanding of the pathogen, host responses, and clinical manifestations of the disease is required. Recently, the application of cutting-edge technologies to the study of *Mtb* pathogenesis has resulted in significant advances in basic biology, vaccine development, and antibiotic discovery. This leaves us in an exciting era of *Mtb* research in which our understanding of this deadly infection is improving at a faster rate than ever, and renews hope in our fight to end TB. In this review, we reflect on what is known regarding *Mtb* pathogenesis, highlighting recent breakthroughs that will provide leverage for the next leaps forward in the field.

1. GLOBAL HEALTH RELEVANCE

Over 135 years ago, Robert Koch identified *Mycobacterium tuberculosis* (*Mtb*) as the etiologic agent of tuberculosis (TB), and the World Health Organization first declared TB a global emergency in 1993. TB disease continues to cause close to 2 million deaths a year, and in 2018 alone, 10 million people fell ill from *Mtb* infection (1). Infection with *Mtb* is monitored by seroconversion based on a positive T cell response to *Mtb* antigens in a tuberculin skin test or interferon gamma (IFN-γ) release assay (IGRA). Following seroconversion, an untreated immunocompetent individual has a 10% lifetime risk of developing active TB, meaning the person will experience the clinical symptoms of TB. Seropositive individuals who do not develop active TB are often considered to have latent TB infection (LTBI); however, there is no diagnostic test that can differentiate between latent versus cleared infection, where immunoreactivity does not reflect infection (2).

2. *MTB*: THE ORGANISM

Mtb is an obligate pathogen of warm-blooded mammals, primarily humans, that belongs to a group of genetically and physiologically similar mycobacterial species that can all cause TB, collectively termed the *Mtb* complex (MTBC). *Mtb* is responsible for most TB cases, with the other members of the MTBC being *Mycobacterium africanum*, *M. bovis*, *M. microti*, *M. canettii*, *M. caprae*, *M. pinnipedii*, and *M. mungi*. MTBC clinical isolates can be classified into seven major lineages based on large sequence polymorphisms, with lineages 2 and 4 being the most geographically widespread (3). These lineages display a degree of geographic restriction, may have different virulence profiles, and can have differences in cell envelope lipid composition (3). A defining feature of *Mtb* is the cell envelope (4). Outside the mycobacterial plasma membrane is a large complex of peptidoglycan covalently linked to arabinogalactan and esterified to α-alkyl, β-hydroxy long-chain (C_{60}–C_{90}) mycolic acids. Lipids and lipoglycans including phosphatidylinositol mannosides (PIMs), phthiocerol dimycocerosates (PDIMs), phenolic glycolipids (PGLs), trehalose dimycolate (TDM), and mannose-capped lipoarabinomannan (ManLAM) are noncovalently intercalated in the mycolic acid layer, and outside this mycomembrane is a loosely attached network of polysaccharides and proteins that collectively form a capsular structure. This unique cell envelope contributes to the high lipid content of *Mtb*, which constitutes 40–60% of the dry cell weight, and is required for virulence, is antigenic and immunomodulatory, and confers resistance to many antibiotics and host-derived stresses (4). The cell envelope also confers the ability of *Mtb* to come together in rope-like cords in the absence of detergent in a process termed cording, which has been associated with virulence (5), inducing necrosis of host cells (6), and the formation of cavitary lesions in humans (5).

The ability to secrete proteins into the host cell is also key to the success of *Mtb* as a pathogen. *Mtb* uses multiple secretion systems to transport proteins across the bacterial plasma membrane, including the SecA1 pathway, the SecA2 pathway, the twin arginine translocation (TAT) system, and five different type VII secretion systems (T7SS) (7). SecA1 and SecA2 enable secretion of unfolded peptides through the SecYEG translocon. SecA1 is required for housekeeping secretion and is essential for survival of the bacteria, whereas SecA2 is required for secretion of a smaller set of proteins, including important virulence factors. The TAT system is also essential for *Mtb* viability and secretes proteins with a conserved N-terminal motif containing two arginine residues. The T7SS, termed ESX-1 through ESX-5, are each encoded in a multigene locus and contribute to various aspects of *Mtb* physiology, with ESX-1, ESX-3, and ESX-5 being shown to contribute to virulence. ESX-1, the first of these systems to be described, is located in the region of difference (RD1) that is absent in the attenuated vaccine strain *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) (8). ESX-1 secretes the virulence factor EsxA (ESAT-6) as a heterodimer with EsxB

(CFP-10) (9). T7SS can also secrete Esp proteins and a family of proline- and glutamate-rich proteins termed PE and PPE proteins, many of which contain a WXG amino acid motif and adopt a four-helix bundle that is characteristic of T7SS substrates (9). T7SS substrates play a myriad of roles in virulence and the physiology of the bacteria. For example, PE5/PPE4 have been shown to mediate iron acquisition (10), and some PE/PPE proteins function as porins to promote nutrient transport across the mycobacterial envelope (11).

3. THE COURSE OF *MTB* INFECTION

Mtb is spread via airborne particles expelled from a person with active pulmonary TB disease. Inhaled bacteria enter the lungs, travel to the alveoli, and are phagocytosed by alveolar macrophages. After phagocytosing the bacteria, the alveolar macrophages cross the lung epithelium into the interstitium (12, 13). The bacteria replicate in the alveolar macrophages and spread through cell lysis to infect other innate immune cells that have been recruited to the site of infection. The bacilli continue to replicate until a cell-mediated immune response occurs (14). This cell-mediated immune response is initiated when monocyte-derived dendritic cells acquire antigen and traffic to the draining lymph node to assist resident lymph node dendritic cells in activating antigen-specific T cells (15). Activated T cells are recruited to the infected lung and form a cuff around the infected focus, leading to the formation of a granuloma. The infection may be cleared; contained to result in LTBI; or cause active TB disease, after which most immunocompetent individuals clear or contain the infection. Although the understanding of protective immunity against *Mtb* infection is incomplete, T lymphocytes, IFN-γ, tumor necrosis factor alpha (TNF-α), and interleukin-12 (IL-12) are all required for prevention of active TB disease; anti-TNF-α therapy is associated with increased reactivation of LTBI (16), and patients with defects in IL-12 or IFN-γ signaling are more susceptible to mycobacterial infections (17). However, individuals with robust T lymphocyte, TNF-α, IFN-γ, and IL-12 responses can also develop active TB disease, indicating that other immune pathways are also critical in controlling *Mtb* infection.

4. THE TB GRANULOMA

Granulomas are complex aggregates of immune cells that form in response to persistent inflammatory stimuli and are the primary setting of pulmonary *Mtb* infection. TB granulomas display a spectrum of phenotypes but can be broadly categorized into several types of lesions, including fibrotic, caseous, or nonnecrotizing (18). Different lesion types are associated with different degrees of success in controlling the infection: Fibrotic lesions are associated with successful immune control with low bacterial burdens, and caseous lesions are associated with poor bacterial killing (18, 19). Nonnecrotic granulomas are found in animals with active disease and are composed primarily of macrophages with few lymphocytes (18). Caseous granulomas are characterized by a central, acellular necrotic core, called a caseum, surrounded by layers of differentiated macrophages and an outer lymphocyte cuff, predominantly composed of $CD4^+$ T cells (20, 21). Other cell types, such as neutrophils and dendritic cells, can also be found throughout caseous granulomas (22). Caseous granulomas often contain collagen surrounding the lymphocyte cuff, in contrast to sterile fibrotic lesions that are typically centrally fibrotic with collagen throughout (19). Extensive necrosis of the inner caseum of granulomas can cause pneumonia, cavitation, and release of bacteria into the airway (23). The walls of cavitary lesions from resected human lungs contain a higher abundance of $FOXP3^+$ lymphocytes compared to granulomatous lesions (21), consistent with a noninflammatory environment. However, these cavity walls also contain proinflammatory cytokine mRNA (24), suggesting that cavitation is not simply the result of a repressed inflammatory response.

4.1. Granuloma Formation

Granuloma formation begins with the infection of an alveolar macrophage, generally by a single *Mtb* bacterium (13, 25). Production of proinflammatory cytokines initiates an influx of immune cells (22). The arrival of adaptive immune cells drives activation and cell death of the infected innate immune cells, releasing bacteria that are rapidly rephagocytosed by incoming phagocytes, resulting in cellular expansion of the granuloma (22). Immune cell aggregation is further facilitated by tissue remodeling by matrix metalloproteinase-9, which is secreted by monocytes and epithelial cells in a TNF-α-dependent manner (26). Some macrophages within caseous granulomas differentiate into interdigitated epithelioid cells, multinucleated giant cells, or lipid-rich foam cells (20). Epithelioid macrophages make up the bulk of the intermediate region of caseous granulomas, express epithelial gene modules, and form canonical desmosomes, adherens junctions, and tight junctions with neighboring macrophages (27). Disrupting these junctions in a zebrafish model of *Mycobacterium marinum* infection results in increased neutrophil infiltration in the granuloma and enhanced bacterial clearance, suggesting that this cell layer may physically occlude protective immune responses (27). Multinucleated giant cells are found in fewer numbers in the intermediate region of the granuloma and represent the fusion of multiple macrophages (20). Lipid-filled foamy macrophages are found surrounding the necrotic caseum of granulomas and are suggested to provide a niche for dormant bacteria until they are able to escape and replicate in the necrotic caseum (21, 28). Foamy macrophages developed in vitro display poor killing of intracellular bacteria and express high levels of IL-10, low levels of TNF-α, and low-level surface expression of major histocompatibility complex (MHC) class II receptors (29–31). *Mtb* mycolic acids induce foamy macrophage differentiation by signaling through G protein–coupled receptor GPR109A (30), peroxisome proliferator-activated receptor gamma, and testicular receptor 4 (29). This leads to upregulation of oxidized low-density lipoprotein uptake receptors CD63 and scavenger receptor alpha, as well as increased triglyceride biosynthesis enzymes to form intracellular lipid droplets (LDs). Historically, LDs have been viewed as a nutrient source for intracellular *Mtb* (28); however, alternative models have emerged that suggest *Mtb*-induced LDs favor the host rather than the bacteria (32, 33).

Lymphocytes comprise the outer ring of leukocytes in the caseous granuloma. However, only about 8% of granuloma T cells respond robustly to *Mtb* antigen (34). Poor effector cell function does not seem to be explained by T cell exhaustion, as T cells in *Mtb*-infected macaque granulomas do not express high levels of inhibitory receptors programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte-associated protein 4 (CTLA-4), and lymphocyte activation gene 3 (LAG-3) and are not functionally exhausted (35). Rather, this defect could be a result of the exclusion of T cells from granuloma centers that contain the highest abundance of infected macrophages (36). Neutrophils are also present within some granulomas, where they express a mixture of pro- and anti-inflammatory cytokines and can form tight interactions with T cells in the lymphocyte cuff (37).

4.2. Granuloma Heterogeneity

The granulomas that develop during TB exhibit both inter- and intra-lesion heterogeneity (38). Multiple lesion types can be found within a single infected individual regardless of disease state (19). PET-CT scanning has revealed that lesions can change from one type to another, behave independently, exhibit inconsistent sterilization following antibiotic treatment, and undergo inconsistent reactivation following TNF-α neutralization (39, 40). Even within a single granuloma, there exists environmental heterogeneity across the spatial landscape of the lesion. The microenvironment within human granulomas roughly transitions from being anti-inflammatory to

proinflammatory as one moves inward from the rim to the caseum (41). The granuloma periphery is enriched for anti-inflammatory eicosanoid biosynthesis enzymes cyclooxygenase 1 (COX1) and COX2, while regions closer to and within the caseum are enriched for proinflammatory eicosanoid biosynthesis enzymes. Macrophage activation status follows a similar pattern, where arginase-1 (ARG1)-expressing M2-like cells are more common in the periphery, while nitric oxide synthase 2 (NOS2)-expressing M1-like cells are more common in the intermediate epithelioid layer (42).

Microscopic examination of granulomas excised from TB patients has revealed growth of *Mtb* in macrophages as well as extracellular growth in the liquefied granuloma centers (24, 38). The diverse microenvironments within lesions affect *Mtb* replication, metabolism, and density of bacterial communities and, consequently, their susceptibility to chemotherapy (38). *Mtb* in the necrotic centers of caseous granulomas are exposed to multiple stresses and are generally not replicating (38). The bacteria within the caseum of lesions in nonhuman primates (NHPs) with active disease express the stress-responsive sigma factor SigH (43), indicating that the bacteria are adapting to stress in the host. Due to destruction of the involved tissue, the interior of the granuloma is poorly vascularized relative to the periphery (44), limiting access of antibiotics to this niche. Caseum within the center of necrotic granulomas also limits passive diffusion of antibiotics (45), resulting in regions of subinhibitory antibiotic concentrations and monotherapy within the granuloma, possibly contributing to the selection of drug resistance (46).

5. ANIMAL MODELS TO STUDY TB

Animal models have been essential in deciphering *Mtb* pathogenesis, with each model providing unique advantages and disadvantages (**Table 1**).

5.1. Mouse Model

The mouse is the most commonly used animal to model *Mtb* infection due to the large range of immunologic and genetic tools available, its small size, and its relatively low cost. Most commonly, mice are infected via the aerosol route, by which a dose of approximately 100 bacteria per mouse results in an initial three-week period of bacterial replication within innate immune cells. Eight to nine days following infection, monocyte-derived dendritic cells carrying *Mtb* antigen traffic to the draining lymph node to present antigen to T cells, causing activation and proliferation of antigen-specific T cells. *Mtb*-specific T cells arrive in the lung at 18–19 days postinfection, activating the innate immune cells to control bacterial replication and inflammation, at which point the bacterial burden levels off in the lungs (47). With the goal of better simulating natural human exposure, mice have also been exposed through the aerosol route to ultralow doses of *Mtb*, ranging from 1 to 11 bacteria per mouse. Three to five weeks postinfection, the ultralow-dose model results in high levels of variability in terms of bacterial burden, providing an opportunity to assess mechanisms associated with heterogeneous outcomes of TB (48).

C57BL/6 mice are the most commonly used strain for dissection of *Mtb* pathogenesis due to the number of genetic tools available, whereas BALB/c mice are commonly used for preclinical evaluation of therapeutics. Both mouse strains can survive with high *Mtb* burdens in the lungs without obvious clinical manifestations of disease for over a year (49). Genetic knockout C57BL/6 mice have been instrumental in establishing our current understanding of *Mtb* pathogenesis. However, these mouse models do not exhibit distinguishing features of human pulmonary TB, such as caseating necrosis, cavitary disease, and fibrosis in the lungs (49). More recently, the C3He3B/FeJ mouse strain has been shown to exhibit increased susceptibility to *Mtb* infection and three distinct types of lesions: necrotic, hypoxic lesions with surrounding epithelioid macrophages; areas

Table 1 Strengths, weaknesses, and applications of animal models of *Mtb* infection

Animal model	Strengths	Limitations	Applications
Zebrafish	Inexpensive Large sample size Ease of breeding/handling Genetic tools Whole-animal imaging (embryo) Caseous granulomas (adult) Latency and reactivation (adult)	Can be infected only with *Mycobacterium marinum* No lung structure No adaptive immunity in embryos	Innate immune dynamics via imaging Innate immunity in granuloma development Interplay between host and pathogen during granuloma initiation and formation Drug screening
Mouse: BALB/c, C57BL/6, C3HeB/FeJ (*Kramnik*), and CC/DO	Inexpensive Large sample size Ease of breeding/handling Immunologic/genetic tools Well-characterized disease course Genetic heterogeneity (CC/DO mice only)	No granulomas No transmission	Role of gene expression in disease and bacterial burden Immune response and mechanisms Drug and vaccine evaluation
Guinea pig	Ease of handling Forms granulomas with necrosis Transmission from humans	More susceptible to *Mtb* than humans No genetic tools Limited immunologic reagents No evidence of latency (due to high susceptibility) No transmission between animals	Pathophysiology of TB Drug and vaccine evaluation *Mtb*-induced cough
Rabbit	Forms granulomas with necrosis and cavitation	Requires technical expertise No genetic tools Limited immunologic reagents	Role of cavitary lesions Historical distinction between *Mycobacterium bovis* and *Mtb*
Marmoset	Smaller than macaques Anatomical, physiological, and genetic similarity to humans Forms granulomas with necrosis and cavitation Can develop latency	Requires technical expertise Expensive No genetic tools Limited immunologic reagents More susceptible to *Mtb* than humans	Host response to varying infection doses Drug and vaccine evaluation Preclinical studies
Macaque	Anatomical, physiological, and genetic similarity to humans Immunologic reagents available Forms granulomas with necrosis and cavitation Can develop latency Coinfection with SIV	Technical expertise required Cost prohibitive No genetic tools Ethical concerns Adequate sample size difficult to achieve	Immunologic and host response to varying infection doses Drug and vaccine evaluation

Abbreviations: CC/DO, collaborative cross/diversity outbred; *Mtb*, *Mycobacterium tuberculosis*; SIV, simian immunodeficiency virus; TB, tuberculosis.

of neutrophilic pneumonia; and lesions indistinguishable from those of C57BL/6 mice (50). Thus, C3He3B/FeJ mice can be used to dissect the events involved in the formation of lesions similar to those observed in human TB. The difference in disease outcomes between C57BL/6 mice and C3He3B/FeJ mice has been attributed to the *super susceptibility to tuberculosis* 1 (*Sst1*) locus (51). Mice carrying the $Sst1^s$ allele, such as C3He3B/FeJ mice, are susceptible to *Mtb* infection due to hyperproduction of type I IFN and inhibition of IL-1 signaling due to elevated IL-1 receptor antagonist (IL-1Ra) (52).

Inbred mouse strains provide experimental consistency to define key players in *Mtb* pathogenesis but do not represent the heterogeneity of the human population. As alternatives, collaborative cross (CC) and diversity outbred (DO) mouse collections (53, 54) have been exploited to study *Mtb* pathogenesis in a more genetically diverse population. The CC collection is a large, multiparental, recombinant inbred reference population composed of lines descended from eight divergent strains of mice (53). Mice from CC lines at early stages of inbreeding were used to establish the DO population, which is maintained by a randomized outbreeding strategy (54). *Mtb* infection of CC or DO mice results in heterogeneous susceptibility and disease outcomes dependent on mouse genetics, similar to humans (55, 56). Infections of CC mice led to the identification of the highly susceptible CC042/GeniUnc mouse strain, which suffered from progressive disease and failed to produce IFN-γ in the lungs (56), and the *Itgal* gene, which is necessary for T cell recruitment to the infected lung (57). DO mice infected with *Mtb* can be categorized as supersusceptible, susceptible, or resistant (58). Chemokines CXCL1, CXCL2, and CXCL5 distinguish the susceptibility groups with approximately 80% accuracy, whereas more commonly assessed molecules (IFN-γ, IL-12, IL-2, and IL-10) were less predictive of outcome (58). Therefore, the genetic diversity allowed by the CC and DO mouse collections can reveal links between alleles and disease outcome.

5.2. Rabbit and Guinea Pig Models

Rabbits can clear *Mtb* over time, but this resistance can be overcome by aerosol infection with a high dose of approximately 1,000 bacteria, such that *Mtb* replicates for 4 weeks, after which the bacterial burden plateaus and remains high for 12 additional weeks. Sixteen weeks postinfection, the rabbit develops necrotic and cavitary lesions, and bacterial growth resumes. This progressive pulmonary disease appears to be attributable to inefficient activation of macrophages, a subsequent delay in T cell activation, and a robust Th2 response (59). The formation of cavitary lesions in rabbits following high-dose *Mtb* infection has provided a model for understanding cavitation in humans, which is associated with high levels of transmission.

The guinea pig model for studying TB dates back to the late 1800s, when Robert Koch first infected guinea pigs with *Mtb*, setting the stage for his renowned Koch's postulates. Following a low-dose [20–50 colony-forming units (CFUs)] aerosol infection, *Mtb* replicates logarithmically in the guinea pig lung for the first 2 weeks postinfection. At 4–6 weeks postinfection, replication stabilizes and is maintained until the guinea pigs succumb to infection between 15 and 20 weeks postinfection (60). Within 5–10 days after infection, guinea pigs develop granulomas with central necrotic caseation that can harbor extracellular bacilli (61), providing an opportunity to study necrotic lesion pathology. Studies performed in guinea pigs have also shown that *Mtb*-derived sulpholipid-1 promotes cough through activation of nociceptive neurons and may facilitate bacterial transmission (62).

5.3. Nonhuman Primate Models

Marmosets are a New World monkey species that have gained popularity as infectious disease models due to their small size and similar anatomy, physiology, and drug metabolism to humans. Marmosets infected with *Mtb* by the aerosol route can develop cavitary lesions in the lung (63). Although marmosets are highly susceptible to *Mtb* and succumb to infection by about 70 days postinfection (63), the severity of disease can be manipulated by varying strain and dose.

Macaques are naturally susceptible to *Mtb* infection and can present all features of human disease. Both the cynomolgus macaque (CM) and the rhesus macaque (RM) are good model systems

for human TB. RM are more susceptible to low-dose infections and more readily develop active TB than CM. Accordingly, RM tend to develop LTBI only about 10% of the time, while CM develop LTBI about 40% of the time (64).

5.4. Zebrafish Model of *Mycobacterium marinum* Infection

In addition to the mammalian models of *Mtb* infection, zebrafish serve as a model for studying host responses to mycobacterial infection using *M. marinum*. Zebrafish larvae are transparent and lack an adaptive immune system until adulthood, facilitating the study of innate immune responses to mycobacterial infection. Furthermore, granuloma formation and progression during *M. marinum* disease in adult zebrafish is similar to *Mtb* in humans, in terms of the granuloma becoming caseous with surrounding fibrosis (65).

6. HOST–PATHOGEN INTERACTIONS DURING *MTB* INFECTION

6.1. Immune Recognition and Host Cell Entry

Within the lung, the surface molecules of *Mtb* serve as pathogen-associated molecular patterns (PAMPs) that lead to recognition by host immune surveillance mechanisms through binding to host pathogen recognition receptors (PRRs) on the host cell surface, including toll-like receptors (TLRs) and C-type lectin receptors. TLR2 is a key cell surface exposed PRR that can heterodimerize with coreceptors such as TLR1 and TLR6 or cooperate with accessory receptors such as CD14 and CD36 to recognize a wide array of *Mtb* PAMPs. Depending on the coreceptor or accessory receptor that is involved, TLR2 can recognize *Mtb* lipoproteins as well as the mannose-containing mycobacterial glycolipids PIM, lipomannan (LM), LAM, and ManLAM to induce downstream immune signaling through nuclear factor κB (NF-κB). *Mtb* is also recognized by TLR4. C-type lectin receptors play an important role in recognizing *Mtb* PAMPs: Mannose receptor, Dectin-2, collectin CL-LK, and DC-SIGN can all bind and respond to ManLAM, whereas Mincle and CLECSF8 bind TDM (66). In addition to the direct interactions between *Mtb* surface molecules and host receptors, the bacteria can also be opsonized by a variety of host factors, including complement, IgG, or surfactant protein A, leading to recognition of opsonized bacteria by complement receptors, Fcγ receptor (FcγR), or surfactant protein A receptor, respectively (67). *Mtb* can also antagonize host PRR signaling to inhibit particular signaling pathways. For example, the *Mtb* surface glycolipids PGL and tetraacylated sulfolipid are TLR2 antagonists that bind this receptor and dampen inflammatory cytokine production (68, 69).

By engaging with host cell receptors, *Mtb* induces receptor-mediated phagocytosis, leading to uptake of the bacteria (66, 67). The receptors that are engaged during phagocytosis impact the intracellular environment that the bacterium experiences. For example, uptake of IgG-coated *Mtb* via FcγR led to more efficient trafficking of the bacteria to lysosomes compared to nonopsonized bacteria, although this did not necessarily lead to more efficient control of bacterial replication (70). In contrast, uptake of *Mtb* through the mannose receptor resulted in inefficient trafficking of the bacterium to lysosomes for degradation (71). Binding of *Mtb* or ManLAM to mannose receptor causes recruitment of the signaling protein Grb2. Grb2 signals through Sos/Rac1/Cdc42 to induce actin remodeling that promotes phagocytosis. However, in addition to inducing phagocytosis, mannose receptor and Grb2 also activate SHP-1, which inhibits key signaling events that are responsible for phagosome maturation and fusion to the lysosome (71). *Mtb* also sheds PDIM into host cell membranes, changing the biophysical properties of the host membranes and stimulating phagocytosis in a receptor-independent manner (72, 73).

6.2. Phagosome Maturation

Once *Mtb* is internalized, the host cell undergoes a series of signaling events that aim to traffic the pathogen to a lysosome for degradation (74). Most studies of intracellular trafficking and signaling in *Mtb*-infected cells have been performed with macrophages, which are the focus of this section. The GTPase Rab5 is present on early phagosomes and recruits effector proteins, including phosphoinositide 3 (PI3) kinase vacuolar protein sorting 34 (VPS34), which decorates the membrane of the maturing phagosome with phosphatidylinositol 3-phosphate (PI3P). Once on the membrane, PI3P binds early endosome antigen 1 (EEA1), an endosomal tethering protein that mediates phagosome-lysosome fusion. As the phagosome matures, Rab5 levels decrease as levels of another GTPase, Rab7, increase in a process termed Rab5-Rab7 conversion. Rab7 then recruits additional effectors such as Rab7-interacting lysosomal protein (RILP), which stimulates transport of the phagosome to the lysosome. During phagosome maturation, vacuolar-associated H^+ ATPase (v-ATPase) and NADPH oxidase are recruited to the phagosomal membrane, which contribute to acidification of the phagosome and production of reactive oxygen species (ROS), respectively.

Despite these mechanisms used to mediate phagosome-lysosome fusion and degradation of phagocytosed cargo, *Mtb* is capable of replicating in macrophages, indicating that the pathogen has ways to subvert these host defenses. Indeed, *Mtb* uses multiple parallel strategies to block virtually every stage of phagosome maturation and thereby promotes its own survival (**Figure 1**). *Mtb* secretes Ndk (nucleotide diphosphate kinase), which acts as a GTPase activating protein (GAP) and inactivates Rab5 and Rab7 to stall phagosome maturation (75). Additionally, *Mtb* inhibits the Rab5-Rab7 conversion, possibly by inducing recruitment of Rab22a to the phagosome, which is proposed to inhibit the fusion with late endosomal vesicles that is required for the Rab5-Rab7 conversion (76). *Mtb* LAM can block the increase in cytosolic Ca^{2+} that is required for the activation of VPS34 (77). Furthermore, *Mtb* secretes SapM (secretory acid phosphatase), which can degrade the PI3P on the surface of the phagosome membrane and prevent phagosome-lysosome fusion mediated through PI3P (78). *Mtb* also secretes the serine/threonine kinase PknG (protein kinase G), which inhibits recruitment of Rab7 and EEA1 through inactivation of Rab29 (79).

Recruitment of v-ATPase to phagosomes containing *Mtb* is also inefficient, in part due to the tyrosine phosphatase PtpA that is secreted from *Mtb* and blocks recruitment of v-ATPase (80). However, a Δ*ptpA* mutant is not attenuated in mice (81), suggesting that either phagosomal acidification does not successfully clear this mutant in vivo or PtpA is not the only strategy used to avoid phagosome acidification. Indeed, PDIM also contributes to exclusion of v-ATPase from the *Mtb*-containing phagosome (72), and *Mtb* secretes 1-tuberculosinyladenosine into the host cell, which acts as an antacid and neutralizes acidic vacuoles in the cell (82). Furthermore, *Mtb* can maintain its intrabacterial pH and survive in the low pH environment of the lysosome (70, 83).

Ligation of surface receptors during pathogen encounter can also trigger LC3-associated phagocytosis (LAP) through the assembly of a VPS34-containing PI3 kinase complex and a NOX2-containing NADPH oxidase complex on the phagosome (84). As part of both of these complexes, Rubicon promotes the sustained production of PI3P and ROS, which leads to recruitment of autophagy related proteins that decorate the phagosome with LC3 (84). *Mtb* secretes CpsA, which prevents localization of NADPH oxidase to *Mtb* phagosomes and inhibits the induction of LAP (85). *Mtb* also antagonizes NADPH oxidase through secretion of Ndk, which has GAP activity against Rac1, a GTPase subunit of NADPH oxidase (86). Secretion of NuoG (NADH dehydrogenase I chain G) and KatG (catalase-peroxidase) by *Mtb* can also neutralize ROS generated by NADPH oxidase (87).

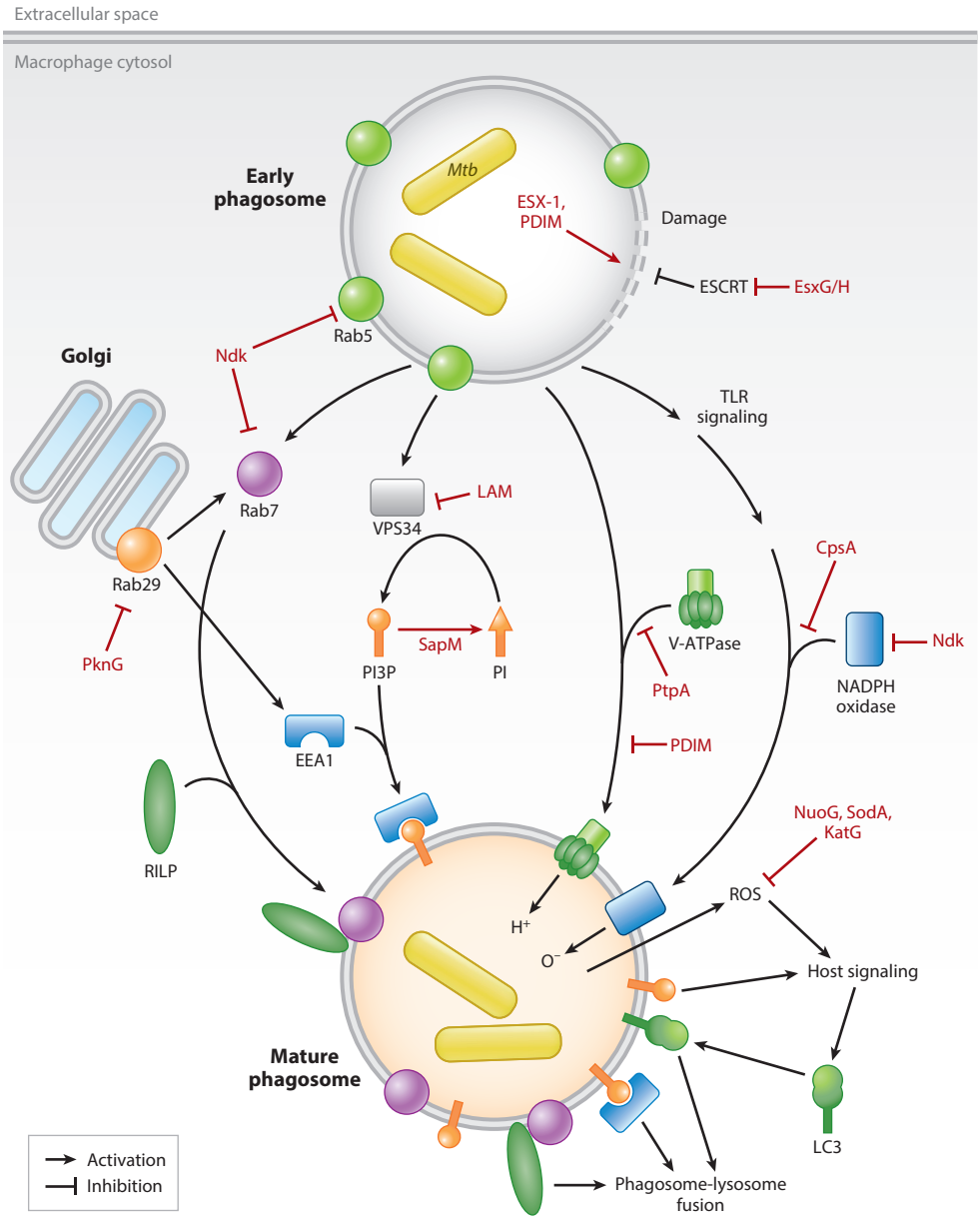

Figure 1

Intracellular trafficking of *Mtb* and bacterial virulence mechanisms. *Mtb* uses many virulence factors (shown in *red text* and *arrows*) that antagonize phagosome maturation and prevent phagosome-lysosome fusion. Abbreviations: EEA1, early endosome antigen 1; ESCRT, endosomal sorting complex required for transport; ESX-1, 6-kDa early secretory antigenic target secretion system 1; KatG, *Mtb* catalase-peroxidase; LAM, lipoarabinomannan; LC3, microtubule-associated proteins 1A/1B light chain 3B; *Mtb*, *Mycobacterium tuberculosis*; Ndk, nucleotide diphosphate kinase; NuoG, NADH dehydrogenase I chain G; PDIM, phthiocerol dimycocerosate; PI, phosphatidylinositol; PI3P, phosphatidylinositol 3-phosphate; PknG, protein kinase G; PtpA, protein tyrosine phosphatase A; RILP, Rab7-interacting lysosomal protein; ROS, reactive oxygen species; SapM, secretory acid phosphatase; SodA, superoxide dismutase A; TLR, Toll-like receptor; V-ATPase, vacuolar-associated H^+ ATPase; VPS34, vacuolar protein sorting 34.

6.3. Phagosomal Membrane Damage and Signaling

Inside the phagosome, *Mtb* secretes effector molecules that damage the phagosomal membrane and signal to cytosolic PRRs. The ESX-1 secretion system is essential to damage the phagosomal membrane, at least in part due to secretion of EsxA (8, 88). EsxA was shown to directly permeabilize membranes; however, this model has recently been questioned due to concerns that EsxA protein preparations were inadvertently contaminated with detergent (88). Detergent-free preparations of EsxA protein lacked membrane permeabilizing activity at neutral pH, and membrane damage by ESX-1 was mediated instead by direct contact of the bacterial cell with the host membrane (88). Recent work has also implicated *Mtb* PDIM in phagosomal membrane damage (89–91); however, loss of PDIM affects secretion of EsxA (91), so the effects on phagosomal membrane integrity may be EsxA dependent and require further study. Upon phagosomal damage, the host ESCRT (endosomal sorting complex required for transport) pathway responds to promote repair of the damaged phagosome (92). However, *Mtb* antagonizes ESCRT-mediated repair through secretion of the ESX-3 substrates EsxG and EsxH (92).

Phagosomal damage allows leakage of *Mtb* PAMPs into the host cell cytosol, which are recognized by cytosolic PRRs. *Mtb* DNA released into the host cell cytosol activates the double-stranded DNA receptor cGAS (cyclic GMP-AMP synthase) (93–96), which synthesizes cyclic GAMP (cGAMP) that signals through the STING/TBK1/IRF3 (stimulator of IFN genes/TANK-binding kinase 1/interferon regulatory factor 3) pathway to activate type I IFN production (95). *Mtb* can also synthesize the cyclic dinucleotide c-di-AMP, which signals directly through STING (97). *Mtb* RNA is transported to the host cytosol in an ESX-1- and SecA2-dependent manner, leading to recognition by RIG-I (retinoic acid–inducible gene I) and signaling through mitochondrial antiviral-signaling protein (MAVS)/TBK1/IRF7 to induce type I IFN production (98). Sensing of *Mtb* peptidoglycan through the cytosolic PRR NOD2 leads to signaling through RIP2/TBK1/IRF5, also inducing type I IFN production (99). TLR9 can also detect *Mtb* DNA and induce proinflammatory cytokine production (66). *Mtb* within damaged phagosomes also signals through the NOD-like receptors (NLRs) NLRP3 or NLRC4 and the cytoplasmic DNA sensor AIM2 (absent in melanoma 2), leading to inflammasome assembly and secretion of the proinflammatory cytokines IL-18 and IL-1β as well as gasdermin D-mediated pyroptotic cell death (94, 100, 101).

These signaling pathways also induce autophagy, leading to the colocalization of ubiquitin, autophagy machinery, and LC3 to a fraction of *Mtb*-containing phagosomes and subsequent formation of double-membrane autophagosomes that are trafficked to the lysosome (95, 96, 102, 103). At least two E3 ligases contribute to ubiquitination of *Mtb*-containing structures in the cell: Parkin (103) and Smurf1 (104). Ubiquitinated *Mtb* phagosomes are recognized by autophagy receptors, including p62, TAX1BP1, optineurin, NBR1, and NDP52 (102, 103). *Mtb* surface protein PE_PGRS29 also harbors a eukaryotic-like ubiquitin binding domain, which facilitates noncovalent interaction of *Mtb* with host ubiquitin chains and triggers delivery of *Mtb* to autophagosomes (105). However, despite some *Mtb* colocalizing with autophagy machinery, *Mtb* employs multiple mechanisms to block autophagy-mediated targeting to the lysosome. *Mtb* secretes Eis, an *N*-acetyltransferase that inhibits autophagy through suppression of ROS (106). *Mtb* promotes the retention of the host factor Coronin-1A on phagosomes, blocking autophagosome formation (107). Mycobacterial ManLAM and secreted PE_PGRS47 protein can also block autophagosome accumulation and maturation through unknown mechanisms (108, 109). Furthermore, macrophages respond to *Mtb* with increased expression of *mir-33*, which downregulates expression of autophagy genes, leading to decreased recruitment of p62 and LC3 to intracellular *Mtb* (110). Given the ability of *Mtb* to block autophagy in the infected cell, loss of autophagy in myeloid cells in the standard

mouse model does not result in susceptibility, although the autophagy-associated protein Atg5 is required to control *Mtb* infection (96, 111, 112) by functioning independently of autophagy to control pathologic neutrophil inflammation (111).

7. TYPE I INTERFERON, INTERLEUKIN-1, AND INTERLEUKIN-10 SIGNALING DURING *MTB* INFECTION

Hypervirulent strains of *Mtb* induce higher levels of type I IFN than less virulent isolates do (113), and leukocytes from active TB patients express higher levels of type I IFN-inducible genes than those from latently infected or uninfected people (114). In addition, most reports involving *Mtb* infection of mice lacking the type I IFN-α/β receptor (IFNAR) or induction of type I IFN signaling during *Mtb* infection support a role for type I IFN signaling in exacerbating *Mtb* infection (113, 115, 116). However, in the absence of IFN-γ signaling, type I IFN provides some protection during *Mtb* infection by preventing anti-inflammatory macrophage polarization (117). In addition, higher bacterial burden early during *Mtb* infection in mice in the absence of IFNAR has been reported (118), suggesting that type I IFN signaling can be protective in certain contexts, which may be related to the overlapping set of interferon stimulated genes (ISGs) for type I IFN and IFN-γ.

Type I IFN signaling through IFNAR activates transcription of >100 ISGs, several of which are upregulated during *Mtb* infection (119). One of the most highly induced ISGs during *Mtb* infection is ISG15 (119), a member of the ubiquitin family that can be conjugated to target proteins in a process termed ISGylation. However, mutations in *ISG15* that prevent its expression are linked to susceptibility to mycobacterial infections in humans (120); in such cases, ISG15 functions intracellularly to stabilize USP18 (ubiquitin-specific protease 18), a potent negative regulator of type I IFN (121) and extracellularly to control *Mtb* infection by synergizing with IL-12 to induce secretion of IFN-γ from T and natural killer (NK) cells (120, 121). For both the extracellular and intracellular activities of ISG15 presented by these studies, conjugation of ISG15 to its targets is not required (120, 121). In contrast to humans, mice deficient in *Isg15* are not susceptible to *Mtb* infection, suggesting *Isg15* is not required for protection in mice and that some aspects of type I IFN responses differ in mice and humans (116).

Type I IFN signaling positively regulates production of the anti-inflammatory cytokine IL-10, which antagonizes IFN-γ-associated pathways (122) (**Figure 2**). Patients with active TB have higher levels of IL-10 in serum and lungs compared to healthy controls (123), where *Mtb* burden in sputum positively correlates with serum IL-10 levels (124), suggesting an association between a loss of bacterial control and higher IL-10 levels. *Il10* expression in T cells and $CD11c^+$ cells is detrimental in the control of chronic *Mtb* infection in mice (125, 126). The transcription factor basic helix-loop-helix family member e40 (Bhlhe40) is required to repress *Il10* expression during *Mtb* infection (126). *BHLHE40* transcript is also reduced in the blood of patients with active TB (114, 126), but whether this directly relates to the higher IL-10 levels in active TB patients is still unknown.

In contrast to type I IFN, IL-1 signaling is required for host defense against *Mtb* infection (127). Mice lacking IL-1R1, IL-1α, or IL-1β succumb to *Mtb* rapidly and have increased bacterial burden and inflammation in the lung (127). Pro-IL-1β is processed by inflammasome-activated caspases 1 or 11 in macrophages but can also be processed in an inflammasome-independent manner by macrophage and neutrophil serine proteases and metalloproteases (128). Caspase 1/caspase 11 and ASC (apoptosis-associated speck-like protein) are critical for inflammasome activation, yet mice deficient in Caspase 1/11 or ASC are not as susceptible to *Mtb* infection as $Il1b^{-/-}$ mice, indicating that inflammasome-independent IL-1β production is essential for control of *Mtb* (127).

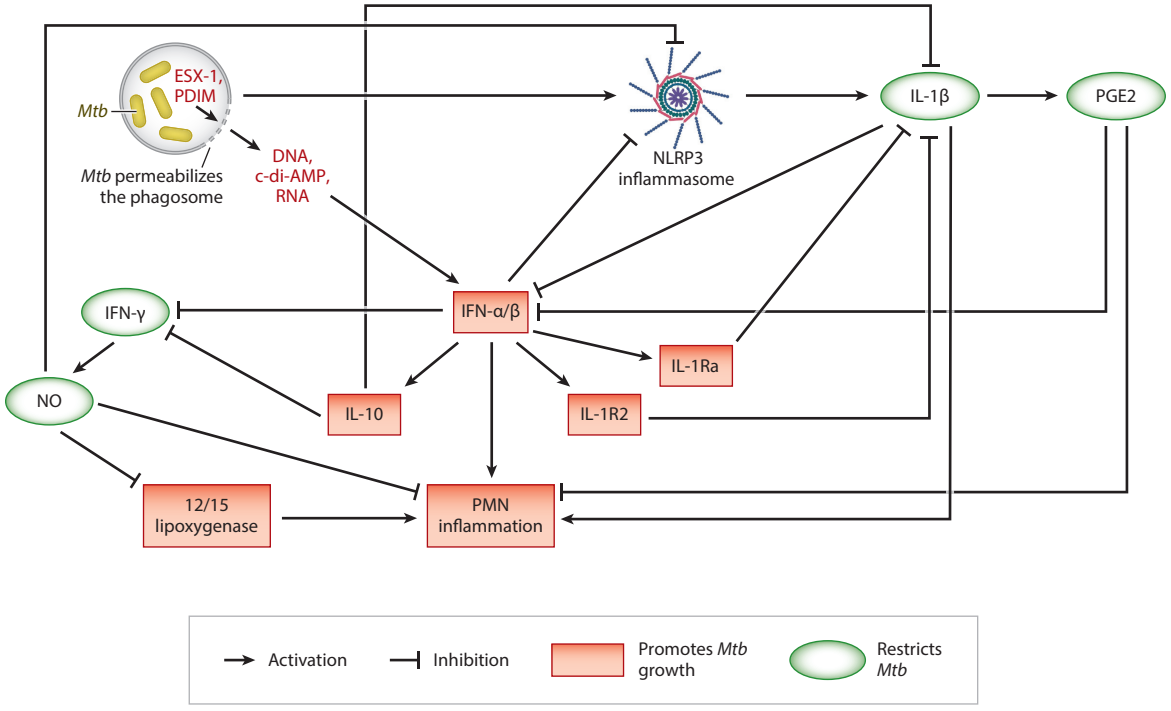

Figure 2

Cross-regulation of IL-1β, type-1 IFN, and IL-10 during immune responses to *Mtb*. *Mtb* factors are shown in red text. Figure adapted from images created with BioRender.com. Abbreviations: ESX-1, 6-kDa early secretory antigenic target secretion system 1; IFN, interferon; IL, interleukin; IL-1R, interleukin-1 receptor; *Mtb*, *Mycobacterium tuberculosis*; NLRP3, NLR family pyrin domain containing 3; NO, nitric oxide; PDIM, phthiocerol dimycocerosate; PGE2, prostaglandin E2; PMN, polymorphonuclear leukocyte.

IL-1 signaling is required to control bacterial spread in phagocytes; however, infected cells lacking IL-1R1 can be protected against infection by IL-1R1 signaling in bystander hematopoietic or nonhematopoietic cells (129). IL-1R1-dependent control of *Mtb* is partially facilitated by COX-2-mediated synthesis of the eicosanoid prostaglandin E2 (PGE2), which limits type I IFN production (130) and protects against mitochondrial damage-induced necrosis (131). Eicosanoids, lipoxins, and leukotrienes are lipid mediators derived from arachidonic acid. In contrast to PGE2, lipoxin A_4 contributes to *Mtb* replication and susceptibility to disease by negatively regulating IL-12 production in mice (132). IL-1-mediated increase of PGE2 limits lipoxin A_4 production, decreasing inflammation (130). As further evidence of the important role for lipid mediators in *Mtb* pathogenesis, polymorphisms that increase expression of human 12/15 lipoxygenase *ALOX12* correlate with increased risk of TB (133). Type I IFN also counterregulates IL-1 signaling by downregulating IL-1α and IL-1β expression, upregulating the IL-1R1 antagonist IL-1Ra, negatively regulating inflammasome activation, and inducing expression of decoy IL-1R2, altogether impairing IL-1-mediated control of *Mtb* (52, 127, 130) (**Figure 2**).

8. HOST CELL DEATH DURING *MTB* INFECTION

An additional consequence of sustained signaling of cytosolic PRRs is host cell death. The mechanism by which an *Mtb*-infected cell dies can impact the subsequent immune responses and control of bacterial replication. Apoptosis is a noninflammatory caspase-8-dependent cell death that

prevents the release of PAMPs and damage-associated molecular patterns (DAMPs). Following apoptosis, the apoptotic *Mtb*-infected cells are taken up by phagocytes through efferocytosis (134). Apoptosis itself is not bactericidal (135), but efferocytosis of *Mtb*-containing apoptotic bodies enhances control of *Mtb* by more efficiently delivering it to the lysosome (135) and promoting efficient antigen presentation during *Mtb* infection (134). In contrast, pyroptosis and necroptosis are lytic forms of cell death that release *Mtb* along with PAMPs and DAMPs, amplifying inflammation and failing to limit bacterial replication (136). Necrophorocytosis—phagocytosis of necrotic phagocytes—promotes bacterial replication and inflammation (137).

Mtb produces several factors that inhibit apoptosis and preferentially induce necrosis. Neutralization of host ROS by *Mtb* effectors NuoG (87), SodA (superoxide dismutase A) (138), and Ndk (86) inhibits apoptosis during infection. Furthermore, *Mtb* can induce the macrophage phosphatase PPM1A (protein phosphatase magnesium-dependent 1A) and secrete Eis into the host cell, which both block apoptosis through inhibition of c-Jun N-terminal kinase (JNK) (139, 140). Instead, *Mtb*-infected macrophages in cell culture and in mice undergo a form of cell death that resembles ferroptosis and promotes bacterial replication (141). Sensing of *Mtb* by NLRP3 can also result in pyroptosis or caspase 1–independent necrotic cell death (142, 143). *Mtb* also produces TB necrotizing toxin (TNT), which hydrolyzes NAD^+ and activates receptor-interacting serine/threonine protein kinase 3 (RIPK3) and necrosis (144). In addition, although TNF-α is required to control *Mtb* infection, high levels of TNF-α can promote necrosis when apoptosis is inhibited, resulting in further inflammation, tissue damage, and loss of bacterial control (145). Deletion of RIPK3 results in lower TNF-α levels, lower *Mtb* lung burden, and reduced immunopathology, which could be explained by a decrease in RIPK3-induced necrosis (145).

9. METABOLIC REGULATION OF IMMUNE RESPONSES TO *MTB*

During *Mtb* infection, macrophages undergo metabolic remodeling, which involves a shift from oxidative phosphorylation (OXPHOS) to glycolysis (146) that is induced by IFN-γ signaling and mediated by the transcription factor hypoxia-inducible factor 1α (HIF-1α) (147). Inhibiting this metabolic change results in reduced IL-1β and increased IL-10, together contributing to increased intracellular survival of *Mtb* (146). As such, *Hif-1α*$^{fl/fl}$*-LysM-Cre* mice fail to initiate a proinflammatory response to *Mtb*, have poor control of bacterial burden, and are severely susceptible to infection (147).

Arginine metabolism is an important regulatory mechanism in macrophage polarization. NOS2 and ARG1 compete for arginine; therefore, ARG1 can inhibit NO production through the depletion of arginine. *Arg1*-deficient macrophages have increased bactericidal activity against *Mtb*, and infection of *Arg1*$^{fl/fl}$*-Tie2-Cre* mice, which lack *Arg1* expression in macrophages, leads to decreased bacterial burden in the lung (148). Alveolar macrophages are less potent at controlling *Mtb* replication than interstitial macrophages due to reduced glycolysis and increased OXPHOS in alveolar macrophages (149). The OXPHOS metabolism in alveolar macrophages is associated with availability of iron and fatty acids, a predominance of fatty acid oxidation, and decreased proinflammatory cytokine production, all resulting in a more permissive environment for *Mtb* (149, 150). In contrast, glycolytic interstitial macrophages limit access to iron through sequestration, produce more NO, and express proinflammatory cytokines (149, 150).

Mtb infection of macrophages leads to upregulation of immune-responsive gene 1 (Irg1), a mitochondrial enzyme that produces itaconate and is required to control proinflammatory responses to limit neutrophil recruitment and prevent susceptibility to *Mtb* infection (151). *Mtb* infection also induces indoleamine 2,3-dioxygenase (IDO) expression, activating tryptophan catabolism, which reduces T cell proliferation, thereby allowing bacterial replication and increased lung pathology

(152). In macaques infected with *Mtb*, blocking IDO results in formation of inducible bronchus-associated lymphoid tissue (iBALT), which is associated with protection from disease, increased T cell proliferation, and T cell localization to the granuloma center (152).

10. INFLAMMATION AND TB

The proinflammatory responses required to control *Mtb* also cause tissue damage and must be precisely balanced with anti-inflammatory responses. In fact, much of the pathology associated with TB may be attributed to the immune response rather than to the bacilli directly. IFN-γ is essential for controlling *Mtb* infection, in part by promoting phagosome maturation and inhibition of bacterial replication in phagocytes, but the susceptibility of IFN-γ-deficient mice has been largely attributed to a failure to limit inflammatory neutrophil recruitment to the infected lung (153). IFN-γ signaling activates macrophage NOS2 to produce NO, which is essential to control *Mtb* infection (133, 154, 155). Although NO has direct bactericidal effects, NOS2 fails to efficiently colocalize with *Mtb*-containing phagosomes in vitro, which is associated with decreased recruitment of the scaffolding protein EBP50 (ezrin-radixin-moesin-binding phosphoprotein 50) that is required for efficient recruitment of NOS2 to phagosomes (156). Instead, NOS2 restricts bacterial replication by limiting neutrophil recruitment through suppression of IL-1β production and restricting ALOX12 (133, 155) (**Figure 2**). NO also represses NF-κB-mediated cytokine expression in macrophages to prevent excessive neutrophil recruitment (154). In addition, *mir-223* and heme oxygenase 1 (HO-1) prevent neutrophil inflammation in response to *Mtb* to alleviate lung pathology (157, 158).

Neutrophil-dominated inflammation has been associated with lack of control of *Mtb* in susceptible mouse models and patients with active TB (58, 111, 114, 153). Neutrophils are the most commonly infected phagocytes in the airways of active TB patients (159) but appear unable to restrict *Mtb* growth, such that neutrophilic inflammation can provide a niche for *Mtb* to survive (111, 133). Neutrophils also release proinflammatory cytokines as well as antimicrobial molecules that can cause tissue damage during *Mtb* infection, further promoting the inflammatory response. Low-density neutrophils (LDNs) in particular have been associated with TB disease progression, as they are present at a higher frequency in the blood of active TB patients compared to healthy individuals (160). LDNs failed to produce ROS and phagocytose *Mtb* in response to stimuli and failed to limit proliferation of antigen-specific T cells, indicating that LDNs may exacerbate TB disease by inhibiting protective adaptive responses (160).

11. T CELLS AND TB

11.1. $CD4^+$ and $CD8^+$ T Cells

$CD4^+$ T cells are critical for the control of *Mtb* in humans and in animal models, in which $CD4^+$ T cell depletion causes reactivation of latent *Mtb* infection (161, 162) and diminished $CD8^+$ T cell cytotoxic activity (163). In particular, IFN-γ from Th1 $CD4^+$ T cells is required to control *Mtb* replication and inflammation (164). Th1 cells contribute to *Mtb* control through TIM-3-galectin-9 (Gal9)-dependent macrophage stimulation, inducing IL-1β production, TNF-α signaling, and macrophage apoptosis, restricting *Mtb* growth (165). In addition, $CD4^+$ T cells have IFN-γ-independent mechanisms of controlling *Mtb* (166, 167). Th2 $CD4^+$ T cells that produce IL-4, IL-5, and IL-13 are ineffective at restricting *Mtb* replication (166) and suppress Th1 responses during infection (168). Th2 responses are also associated with increased incidence of reactivation in latently infected individuals (169). Th17 $CD4^+$ T cells are present in pulmonary lesions of TB patients (170); however, their role during TB is unclear. In mice, IL-17 signaling is required for protection against infection with *Mtb* lineage 2 strain HN878 but not lineage 4 strains (171).

$CD8^+$ T cells respond to *Mtb* infection by producing IFN-γ and secreting the cytolytic enzymes granzyme and perforin, which can kill *Mtb*-infected cells (172). Mice lacking peptide antigen presentation on MHC class I molecules infected intravenously with *Mtb* succumb to infection earlier than control mice do, which correlated with fewer $CD8^+$ T cells in the peripheral blood (173). $CD8^+$ T cell depletion experiments support that $CD8^+$ T cells contribute to *Mtb* growth restriction during chronic infection (174), and transfer of primed *Mtb*-specific $CD8^+$ T cells into irradiated mice conferred better protection against *Mtb* replication compared to nonspecific T cells (175), which required perforin-mediated cytotoxicity (172). Nonetheless, $CD8^+$ T cell responses cannot compensate for loss of $CD4^+$ T cells (164).

The expansion of anti-inflammatory $Foxp3^+$ T regulatory cells (Tregs) during *Mtb* infection suppresses proliferation and activation of Th1 cells (176, 177), contributing to increased bacterial burden in mouse models (176). Tregs are more abundant in active TB patients compared to LTBI patients (178), supporting an association between Tregs and a loss of control of infection. There are also signs of T cell exhaustion during chronic *Mtb* infection, including $CD4^+$ T cells producing lower levels of IL-2, IFN-γ, and TNF-α (179, 180). The expression of T cell coinhibitory receptor PD-1 increases on *Mtb*-specific $CD4^+$ T cells during active TB, suggesting PD-1 may inhibit protective $CD4^+$ T cell responses (179). However, PD-1-deficient mice and mice that receive PD-1 blockade are more susceptible to *Mtb* due to elevated $CD4^+$ T cell numbers in the lung, leading to exaggerated IFN-γ production and lethal immunopathology (180). In addition, PD-1 blockade in cancer patients has been associated with increased risk of active TB (181). Deletion of the mitochondrial protein cyclophilin D, which normally negatively regulates T cell proliferation (182), also results in susceptibility of mice to *Mtb* due to increased $CD8^+$ and $CD4^+$ T cell responses driving lung pathology, further supporting the need for control of inflammatory T cell responses.

11.2. Innate-Like Lymphocytes

Mycolipid antigens that dominate the outer surface of *Mtb* are presented on MHC-like molecules CD1a–d. CD1b-restricted germline-encoded mycolyl lipid-reactive (GEM) T cells and CD1d-restricted natural killer T (NKT) cells are the two CD1-responsive lymphocyte populations best characterized in the context of *Mtb* infection. CD1b is expressed within the granulomas of human TB patients (183), but a functional role for GEM T cells has not been defined. NKT cells can mediate bacterial control in cocultured *Mtb*-infected murine macrophages in vitro through secretion of IFN-γ (184) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (185). However, mice lacking NKT cells are not more susceptible to *Mtb* infection (186). Mucosal-associated invariant T (MAIT) cells are a subpopulation of innate-like $CD8^+$ T cells that express semi-invariant T cell receptor alpha variable 1-2 (TRAV1-2), which recognizes *Mtb* vitamin B–derived antigens presented on the MHC-related protein 1 (MR-1) molecule. MAIT cells are abundant in healthy airway mucosa and produce IFN-γ in response to *Mtb*-infected dendritic cells and lung epithelial cells (187, 188). In TB patients, MAIT cells are depleted from peripheral blood and enriched within the bronchoalveolar lavage fluid (188), suggesting that they accumulate in the lungs during infection. However, the role of MAIT cells during *Mtb* infection is unknown. Vγ9Vδ2 T cells are a subset of γδ T cells that recognize phosphoantigens produced by *Mtb* and are only found within humans and NHPs (189). Primary exposure to *Mtb* induces a massive expansion of Vγ9Vδ2 T cells that exhibit memory-like rapid recall expansion following subsequent challenge with *Mtb* (190). Adoptively transferred Vγ9Vδ2 T cells traffic to the airways of NHPs and provide protection against *Mtb* infection (191), although the mechanism remains unknown.

NK cells accumulate in the lungs of infected mice and can produce IFN-γ (192), promote control of bacterial replication within macrophages (193), kill infected cells (194), and promote γδ T

cell proliferation during *Mtb* infection (195). However, depletion of NK cells from *Mtb*-infected mice does not affect bacterial burdens (192). Group 1 innate lymphoid cells (ILC1s), ILC2s, and ILC3s also respond to *Mtb* infection. ILC1s produce IFN-γ and are considered the innate counterpart of Th1 cells; ILC2s produce IL-4, IL-5, and IL-13, mirroring the function of Th2 cells; and ILC3s are heterogeneous but can produce IL-17, IL-22, or both, mirroring Th17 cells. The levels of ILCs circulating in peripheral blood of active TB patients were reduced compared to healthy individuals, and ILC3s were enriched in iBALT-associated granulomas (196). ILC3s were recruited to the lungs in *Mtb*-infected mice, and depletion of ILC3s resulted in increased bacterial burden at 14 days postinfection and decreased iBALT formation in the lung (196).

12. B CELLS AND TB

B cells are found in cellular aggregates surrounding the lymphoid cuff of granulomas in the lungs of TB patients and *Mtb*-infected NHPs (197). These structures resemble tertiary lymphoid organs called iBALT, containing antigen-presenting cells, plasma cells secreting *Mtb*-specific antibodies, and T cells (197). When iBALT structures are perturbed in mice lacking CXCL13/CXCR5 chemokine signaling, animals show poor lymphocyte organization and poor mycobacterial control in lesions (198). In NHPs, iBALT formation is associated with reduced granuloma area and latent disease (198). Depleting B cells from macaques during the acute phase of *Mtb* infection resulted in increased granuloma CFUs and increased IL-2, IL-10, and IL-17 production by T cells but did not result in any significant changes in granuloma structure (199).

Recent findings have also indicated that antibodies are important players in TB disease. Active TB and LTBI patients can be distinguished based on Fc domain glycosylation patterns and unique glycosylation-dependent functional properties of their serum antibodies (200). Antibodies from LTBI patients bound more strongly to activating FcγRIII, increased NK cell antibody-dependent cellular cytotoxicity, and enhanced macrophage killing of intracellular *Mtb* compared to antibodies from active TB patients. In addition, *Mtb*-specific antibodies were detected in individuals who historically have been termed resisters because they remain IGRA negative despite close contact with active TB patients, indicating that antibodies may provide protection (201). Antibodies from the resister population upregulate CD40L/CD154 in T cells, induce IFN-γ release from NK cells, and display higher antigen binding avidity and a unique glycosylation profile compared to LTBI antibodies.

Human antibodies against *Mtb* confer some protection against *Mtb* infection in mice, which is dependent on Fc domain glycosylation and T cells (202, 203). Mice lacking the inhibitory FcγRIIB showed improved containment of *Mtb*, enhanced Th1 responses, and lower IL-10 levels in the lung, while mice lacking the common γ-chain shared by all activating FcγRs showed increased IL-10, neutrophils, and bacterial CFUs in the lung (204), further supporting a role for antibody-FcγR interactions. However, the Fab fragment of a LAM-targeting antibody was also able to confer some protection against *Mtb* infection in mice (205). Furthermore, passive transfer of hyperimmune serum raised in an *Mtb*-infected mouse was able to protect SCID mice from *Mtb* infection (206). Together, this suggests antibodies can be protective in the absence of T cells or in an Fc-independent mechanism.

13. TB COMORBIDITIES

13.1. HIV

Individuals infected with HIV are 15–22 times more likely to develop TB, and in 2018, HIV-associated TB was responsible for 251,000 deaths (1) (**Figure 3**). Depletion of $CD4^+$ T cells during HIV infection is a key contributor to increased susceptibility to developing active TB disease

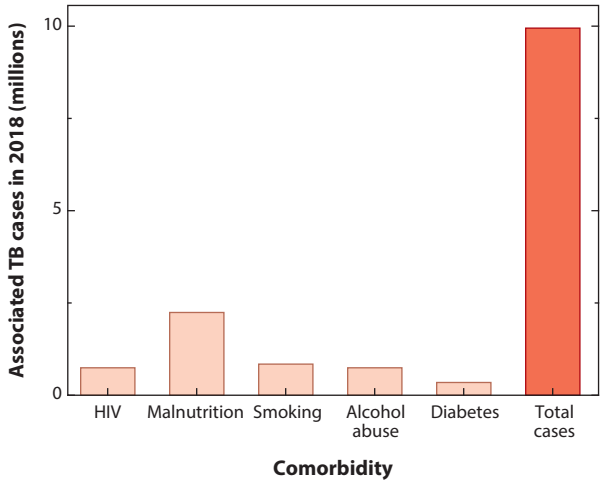

Figure 3

Number of 2018 TB cases associated with key comorbidities. Abbreviation: TB, tuberculosis.

(162). The role of $CD8^+$ T cells is more complex: $CD8^+$ T cell proliferation correlated with control of LTBI in macaques coinfected with simian immunodeficiency virus (207), but people coinfected with HIV and *Mtb* have reduced numbers of *Mtb*-specific $CD8^+$ T cells and increased PD-1 expression on T cells (208). HIV also infects macrophages, and coinfection of macrophages in vitro with HIV and *Mtb* results in increased bacterial and viral replication and dysregulated proinflammatory signaling (209). TB immune reconstitution inflammatory response syndrome (TB-IRIS) can also occur in TB patients beginning antiretroviral treatment (ART). TB-IRIS involves a hyperinflammatory response that is thought to be caused by a rebound in both the innate and adaptive immune system due to reduced HIV burden and HIV suppression of immune mechanisms (210). One of the most predictive risk factors for TB-IRIS is low $CD4^+$ T cell counts prior to initiating ART and successful increases in $CD4^+$ T cell counts during treatment (210). However, many patients who experience increased $CD4^+$ T cell counts during ART do not develop IRIS, indicating other contributors to TB-IRIS exist. Another potential mechanism underpinning IRIS is Treg dysfunction. Tregs collected from the blood of TB-IRIS patients showed decreased ability to suppress production of proinflammatory cytokines and produced less IL-10 in vitro (211). Other studies have identified an association between higher plasma levels of IL-6, C reaction protein, IFN-γ, TNF-α, IL-12, and IFN-α prior to ART as being associated with developing TB-IRIS (210).

13.2. Diabetes

Diabetes mellitus is increasing in prevalence globally, and 400,000 TB cases in 2018 were associated with diabetes (1) (**Figure 3**). In addition, TB can worsen glycemic control in people with diabetes. Although both type 1 and type 2 diabetes (T2D) are associated with increased susceptibility to TB, T2D is more prevalent. T2D-induced mice succumb earlier to *Mtb* infection compared to non-T2D mice (212). The increased risk of TB likely stems from the metabolic and inflammatory consequences of chronic hyperglycemia (213). Alveolar macrophages from the lungs of T2D-induced mice and macrophages from T2D patients have reduced ability to phagocytose *Mtb*, which is attributed to the decreased expression of surface receptors important for recognizing and engulfing the bacteria (214). Macrophages exposed to high glucose levels accumulate

higher levels of oxidized low-density lipids and are less capable of controlling *Mtb* replication due to lysosomal dysfunction (215). Resistin, a protein that decreases insulin sensitivity, is elevated in T2D and has been shown to decrease the production of ROS by macrophages in response to *Mtb* infection (216). In addition to macrophages, the total number of neutrophils is increased in TB patients with T2D, and neutrophils from these individuals are less capable of phagocytosing *Mtb* (217). TB patients with T2D have lower levels of IL-22 compared to TB patients without T2D, and recombinant IL-22 treatment or adoptive transfer of ILC3s that make IL-22 into T2D-induced mice infected with *Mtb* improved survival of the mice, decreased neutrophil accumulation, and decreased epithelial damage in the lungs (218). T2D-induced mice also exhibit a delay in the proliferation and migration of T cells to sites of *Mtb* infection (219).

13.3. Smoking, Alcohol Abuse, and Malnutrition

Close to one million TB cases were associated with smoking in 2018 (1) (**Figure 3**). Mice exposed to cigarette smoke and then infected with *Mtb* exhibited decreased IFN-γ-positive T cells in the lung, increased lung bacterial burden, and decreased survival (220). Macrophages exposed to cigarette smoke develop a vacuolated morphology with debris accumulating in large lysosomal inclusion bodies (221) and produce less TNF-α, IFN-γ, and IL-1β when infected with mycobacteria (222). Approximately 800,000 TB cases in 2018 were associated with alcohol abuse (1) (**Figure 3**). Alcohol has immunosuppressive effects, and ethanol-fed mice infected with *Mtb* have higher bacterial burdens, smaller immune cell aggregates in the lung, increased type I IFN levels in the lung, increased macrophage necroptosis, decreased T cell proliferation and production of IFN-γ, and decreased survival, where IFNAR blockade improves survival (223, 224). Malnutrition is a serious public health problem worldwide, and studies suggest a 6–10-fold increased risk of developing active TB when malnourished (225). Although there is extensive evidence of immune dysfunction as a consequence of malnutrition (226), the mechanisms of TB susceptibility are unclear. Nonetheless, in its 2019 TB report, the World Health Organization cited improved nutrition as a sustainable goal that would improve overall global health as well as reduce the TB burden (1).

14. TREATMENT OF TB

The standard treatment for drug-sensitive cases of active TB consists of an intensive phase of 2 months with daily dosing of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB) (or streptomycin), followed by a 4-month continuation phase of INH and RIF. This long, 6-month regimen is associated with many side effects, which often results in poor patient compliance. Poor adherence to treatment regimens increases the risk of disease relapse and development of antibiotic-resistant TB. In 2017, an estimated 19% of newly treated cases and 43% of previously treated cases exhibited resistance to INH or RIF (1). INH is a prodrug that is converted to its active form by the *Mtb* catalase peroxidase KatG, which adds an NAD^+ adduct to INH (INH-NAD) (227). The most well-characterized target of INH-NAD is the essential mycolic acid biosynthesis enzyme InhA; however, other targets for INH-NAD have also been described. The most common mechanism of INH resistance occurs through mutations in *katG*; over 300 different mutations in *katG* have been reported in INH-resistant clinical isolates of *Mtb* (**Figure 4**). Increased INH resistance can also occur through mutations that decrease *katG* expression, increase *inhA* expression, or decrease INH-NAD binding to InhA (227). INH resistance is associated with treatment failure, relapse, and progression to multidrug-resistant TB (MDR-TB), defined as TB resistant to at least INH and RIF. RIF targets the RNA polymerase β subunit (RpoB) and prevents transcription (228) (**Figure 4**). Mutations in the drug target *rpoB* are responsible for over 90% of

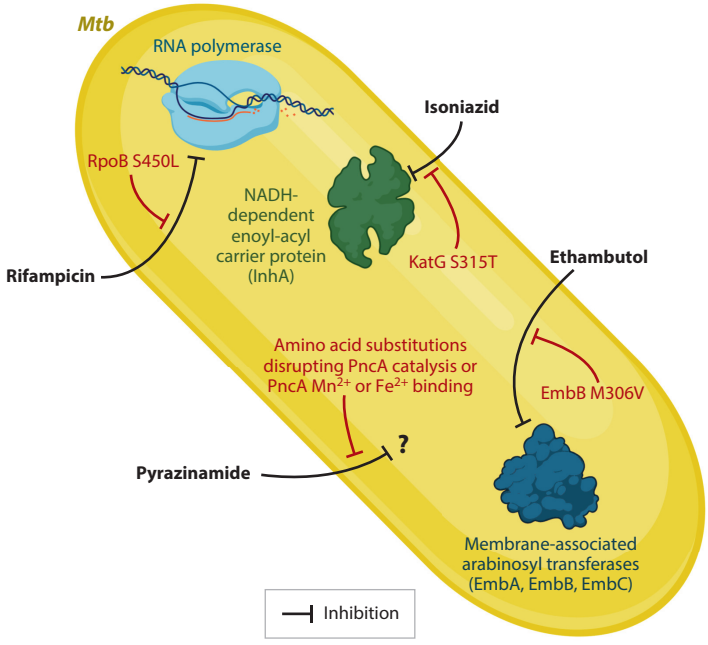

Figure 4

Molecular targets of frontline TB antibiotics and associated resistance mechanisms. The most common resistance mutations are shown in red. Figure adapted from images created with BioRender.com. Abbreviations: EMB, ethambutol; INH, isoniazid; KatG, *Mtb* catalase-peroxidase; *Mtb*, *Mycobacterium tuberculosis*; PncA, pyrazinamidase; RpoB, RNA polymerase β subunit; TB, tuberculosis.

RIF resistance in clinical isolates (229). The majority of the *rpoB* mutations are located in or near the RIF binding pocket in RpoB and interfere with RIF binding (228). RIF-resistant *Mtb* clinical isolates without mutations in *rpoB* have been reported but are rare, and the mechanisms are not yet fully understood (229). Patients with RIF-resistant or MDR-TB are treated with a combination of second-line drugs associated with significant toxicity and a clinical cure rate of only 55%. In 2017, 8.5% of MDR-TB cases were extensively drug-resistant TB (XDR-TB), defined as MDR-TB with additional resistance to a fluoroquinolone as well as a second-line injectable drug (1). The prognosis of XDR-TB is extremely poor, with few resources left for treatment.

Resistance to PZA and EMB has also been isolated clinically. PZA is a prodrug that is converted to its active form, pyrazinoic acid, by the amidase pyrazinamidase encoded by *pncA*, which is involved in the NAD^+ salvage pathway but is nonessential (230). Most PZA resistance in *Mtb* clinical isolates is attributed to mutations in *pncA*; however, resistant isolates without mutations in *pncA* indicate that other resistance mechanisms are clinically relevant (230) (**Figure 4**). EMB inhibits the biosynthesis of the cell wall component arabinogalactan (231), most likely by inhibiting EmbB, which is a transmembrane-spanning glycosyltransferase involved in arabinan synthesis (232). Up to 70% of EMB-resistant clinical isolates have mutations in *embB* (232). In addition to *embB*, the other two genes that comprise the *embBAC* operon, which are also glycosyltransferases and likely involved in arabinan or lipoarabinan biosynthesis, can be mutated to confer EMB resistance (233) (**Figure 4**). Additionally, mutations in the promoter and open reading frame of the transcription factor that controls *embBAC* expression, EmbR, are also commonly found in EMB resistant isolates (234).

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors thank members of the Stallings lab for their careful reading of drafts and suggestions. C.L.S. is supported by the National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID) grants AI132653, AI132697, AI142784, AI111696, and AI134847 and by a Burroughs Wellcome Fund Investigators in the Pathogenesis of Infectious Disease award. R.L.K. is supported by a Potts Memorial Foundation postdoctoral fellowship. G.A.H. is supported by the National Science Foundation Graduate Research Fellowship Program award DGE-1745038 and by the NIH/National Institute of General Medical Sciences grant GM007067. D.X.Z. is supported by the NIH/NIAID grant A1007172.

LITERATURE CITED

1. WHO (World Health Organ.). 2019. *Global Tuberculosis Report 2019*. Geneva: WHO. **https://www.who.int/tb/publications/global_report/en/**
2. Behr MA, Edelstein PH, Ramakrishnan L. 2019. Is *Mycobacterium tuberculosis* infection life long? *BMJ* 367:l5770
3. Coscolla M, Gagneux S. 2014. Consequences of genomic diversity in *Mycobacterium tuberculosis*. *Semin. Immunol.* 26(6):431–44
4. Jackson M. 2014. The mycobacterial cell envelope—lipids. *Cold Spring Harb. Perspect. Med.* 4(10):a021105
5. Hunter RL, Olsen MR, Jagannath C, Actor JK. 2006. Multiple roles of cord factor in the pathogenesis of primary, secondary, and cavitary tuberculosis, including a revised description of the pathology of secondary disease. *Ann. Clin. Lab. Sci.* 36(4):371–86
6. Kalsum S, Braian C, Koeken VACM, Raffetseder J, Lindroth M, et al. 2017. The cording phenotype of *Mycobacterium tuberculosis* induces the formation of extracellular traps in human macrophages. *Front. Cell. Infect. Microbiol.* 7:278
7. Feltcher ME, Sullivan JT, Braunstein M. 2010. Protein export systems of *Mycobacterium tuberculosis*: novel targets for drug development? *Future Microbiol.* 5(10):1581–97
8. Hsu T, Hingley-Wilson SM, Chen B, Chen M, Dai AZ, et al. 2003. The primary mechanism of attenuation of bacillus Calmette–Guérin is a loss of secreted lytic function required for invasion of lung interstitial tissue. *PNAS* 100(21):12420–25
9. Abdallah AM, Gey van Pittius NC, DiGiuseppe Champion PA, Cox J, Luirink J, et al. 2007. Type VII secretion—mycobacteria show the way. *Nat. Rev. Microbiol.* 5(11):883–91
10. Tufariello JM, Chapman JR, Kerantzas CA, Wong K-W, Vilchèze C, et al. 2016. Separable roles for *Mycobacterium tuberculosis* ESX-3 effectors in iron acquisition and virulence. *PNAS* 113(3):E348–57
11. Wang Q, Boshoff HIM, Harrison JR, Ray PC, Green SR, et al. 2020. PE/PPE proteins mediate nutrient transport across the outer membrane of *Mycobacterium tuberculosis*. *Science* 367(6482):1147–51
12. Dunlap MD, Howard N, Das S, Scott N, Ahmed M, et al. 2018. A novel role for C-C motif chemokine receptor 2 during infection with hypervirulent *Mycobacterium tuberculosis*. *Mucosal Immunol.* 11(6):1727–42
13. Cohen SB, Gern BH, Delahaye JL, Adams KN, Plumlee CR, et al. 2018. Alveolar macrophages provide an early *Mycobacterium tuberculosis* niche and initiate dissemination. *Cell Host Microbe* 24(3):439–46.e4
14. Ahmad S. 2011. Pathogenesis, immunology, and diagnosis of latent *Mycobacterium tuberculosis* infection. *Clin. Dev. Immunol.* 2011:814943
15. Srivastava S, Ernst JD. 2014. Cell-to-cell transfer of *M. tuberculosis* antigens optimizes CD4 T cell priming. *Cell Host Microbe* 15(6):741–52

16. Cantini F, Niccoli L, Goletti D. 2014. Tuberculosis risk in patients treated with non-anti-tumor necrosis factor-α (TNF-α) targeted biologics and recently licensed TNF-α inhibitors: data from clinical trials and national registries. *J. Rheumatol.* 41(Suppl. 91):56–64
17. van de Vosse E, Hoeve MA, Ottenhoff THM. 2004. Human genetics of intracellular infectious diseases: molecular and cellular immunity against mycobacteria and salmonellae. *Lancet Infect. Dis.* 4(12):739–49
18. Lin PL, Rodgers M, Smith L, Bigbee M, Myers A, et al. 2009. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. *Infect. Immun.* 77(10):4631–42
19. Lin PL, Ford CB, Coleman MT, Myers AJ, Gawande R, et al. 2014. Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. *Nat. Med.* 20(1):75–79
20. McClean CM, Tobin DM. 2016. Macrophage form, function, and phenotype in mycobacterial infection: lessons from tuberculosis and other diseases. *Pathog. Dis.* 74(7):ftw068
21. Welsh KJ, Risin SA, Actor JK, Hunter RL. 2011. Immunopathology of postprimary tuberculosis: increased T-regulatory cells and DEC-205-positive foamy macrophages in cavitary lesions. *Clin. Dev. Immunol.* 2011:307631
22. Pagán AJ, Ramakrishnan L. 2015. Immunity and immunopathology in the tuberculous granuloma. *Cold Spring Harb. Perspect. Med.* 5(9):a018499
23. Hunter RL. 2016. Tuberculosis as a three-act play: a new paradigm for the pathogenesis of pulmonary tuberculosis. *Tuberculosis* 97:8–17
24. Subbian S, Tsenova L, Kim MJ, Wainwright HC, Visser A, et al. 2015. Lesion-specific immune response in granulomas of patients with pulmonary tuberculosis: a pilot study. *PLOS ONE* 10(7):e0132249
25. Martin CJ, Cadena AM, Leung VW, Lin PL, Maiello P, et al. 2017. Digitally barcoding *Mycobacterium tuberculosis* reveals in vivo infection dynamics in the macaque model of tuberculosis. *mBio* 8(3):e00312–17
26. Price NM, Gilman RH, Uddin J, Recavarren S, Friedland JS. 2003. Unopposed matrix metalloproteinase-9 expression in human tuberculous granuloma and the role of TNF-α-dependent monocyte networks. *J. Immunol.* 171(10):5579–86
27. Cronan MR, Beerman RW, Rosenberg AF, Saelens JW, Johnson MG, et al. 2016. Macrophage epithelial reprogramming underlies mycobacterial granuloma formation and promotes infection. *Immunity* 45(4):861–76
28. Russell DG, Cardona P-J, Kim M-J, Allain S, Altare F. 2009. Foamy macrophages and the progression of the human tuberculosis granuloma. *Nat. Immunol.* 10:943
29. Mahajan S, Dkhar HK, Chandra V, Dave S, Nanduri R, et al. 2012. *Mycobacterium tuberculosis* modulates macrophage lipid-sensing nuclear receptors PPARγ and TR4 for survival. *J. Immunol.* 188(11):5593–603
30. Singh V, Jamwal S, Jain R, Verma P, Gokhale R, Rao KVS. 2012. *Mycobacterium tuberculosis*-driven targeted recalibration of macrophage lipid homeostasis promotes the foamy phenotype. *Cell Host Microbe* 12(5):669–81
31. Genoula M, Franco JLM, Dupont M, Kviatcovsky D, Milillo A, et al. 2018. Formation of foamy macrophages by tuberculous pleural effusions is triggered by the interleukin-10/signal transducer and activator of transcription 3 axis through ACAT upregulation. *Front. Immunol.* 9:459
32. Knight M, Braverman J, Asfaha K, Gronert K, Stanley S. 2018. Lipid droplet formation in *Mycobacterium tuberculosis* infected macrophages requires IFN-γ/HIF-1α signaling and supports host defense. *PLOS Pathog.* 14(1):e1006874
33. Jaisinghani N, Dawa S, Singh K, Nandy A, Menon D, et al. 2018. Necrosis driven triglyceride synthesis primes macrophages for inflammation during *Mycobacterium tuberculosis* infection. *Front. Immunol.* 9:1490
34. Gideon HP, Phuah JY, Myers AJ, Bryson BD, Rodgers MA, et al. 2015. Variability in tuberculosis granuloma T cell responses exists, but a balance of pro- and anti-inflammatory cytokines is associated with sterilization. *PLOS Pathog.* 11(1):e1004603
35. Wong EA, Evans S, Kraus CR, Engelman KD, Maiello P, et al. 2020. IL-10 impairs local immune response in lung granulomas and lymph nodes during early *Mycobacterium tuberculosis* infection. *J. Immunol.* 204(3):644–59
36. Kauffman KD, Sallin MA, Sakai S, Kamenyeva O, Kabat J, et al. 2018. Defective positioning in granulomas but not lung-homing limits CD4 T-cell interactions with *Mycobacterium tuberculosis*-infected macrophages in rhesus macaques. *Mucosal Immunol.* 11(2):462–73

37. Gideon HP, Phuah J, Junecko BA, Mattila JT. 2019. Neutrophils express pro- and anti-inflammatory cytokines in granulomas from *Mycobacterium tuberculosis*-infected cynomolgus macaques. *Mucosal Immunol.* 12(6):1370–81
38. Lenaerts A, Barry CE III, Dartois V. 2015. Heterogeneity in tuberculosis pathology, microenvironments and therapeutic responses. *Immunol. Rev.* 264(1):288–307
39. Lin PL, Maiello P, Gideon HP, Coleman MT, Cadena AM, et al. 2016. PET CT identifies reactivation risk in cynomolgus macaques with latent *M. tuberculosis*. *PLOS Pathog.* 12(7):e1005739
40. Malherbe ST, Shenai S, Ronacher K, Loxton AG, Dolganov G, et al. 2016. Persisting positron emission tomography lesion activity and *Mycobacterium tuberculosis* mRNA after tuberculosis cure. *Nat. Med.* 22(10):1094–100
41. Marakalala MJ, Raju RM, Sharma K, Zhang YJ, Eugenin EA, et al. 2016. Inflammatory signaling in human tuberculosis granulomas is spatially organized. *Nat. Med.* 22(5):531–38
42. Mattila JT, Ojo OO, Kepka-Lenhart D, Marino S, Kim JH, et al. 2013. Microenvironments in tuberculous granulomas are delineated by distinct populations of macrophage subsets and expression of nitric oxide synthase and arginase isoforms. *J. Immunol.* 191(2):773–84
43. Hudock TA, Foreman TW, Bandyopadhyay N, Gautam US, Veatch AV, et al. 2017. Hypoxia sensing and persistence genes are expressed during the intragranulomatous survival of *Mycobacterium tuberculosis*. *Am. J. Respir. Cell Mol. Biol.* 56(5):637–47
44. Aly S, Laskay T, Mages J, Malzan A, Lang R, Ehlers S. 2007. Interferon-gamma-dependent mechanisms of mycobacteria-induced pulmonary immunopathology: the role of angiostasis and CXCR3-targeted chemokines for granuloma necrosis. *J. Pathol.* 212(3):295–305
45. Sarathy JP, Zuccotto F, Hsinpin H, Sandberg L, Via LE, et al. 2016. Prediction of drug penetration in tuberculosis lesions. *ACS Infect. Dis.* 2(8):552–63
46. Strydom N, Gupta SV, Fox WS, Via LE, Bang H, et al. 2019. Tuberculosis drugs' distribution and emergence of resistance in patient's lung lesions: a mechanistic model and tool for regimen and dose optimization. *PLOS Med.* 16(4):e1002773
47. Muñoz-Elías EJ, Timm J, Botha T, Chan WT, Gomez JE, McKinney JD. 2005. Replication dynamics of *Mycobacterium tuberculosis* in chronically infected mice. *Infect. Immun.* 73(1):546–51
48. Gern B, Plumlee C, Gerner M, Urdahl K. 2017. Investigating immune correlates of protection to tuberculosis using an ultra-low dose infection in a mouse model. *Open Forum Infect. Dis.* 4(Suppl. 1):S47–48
49. Orme IM. 2003. The mouse as a useful model of tuberculosis. *Tuberculosis* 83(1–3):112–15
50. Irwin SM, Driver E, Lyon E, Schrupp C, Ryan G, et al. 2015. Presence of multiple lesion types with vastly different microenvironments in C3HeB/FeJ mice following aerosol infection with *Mycobacterium tuberculosis*. *Dis. Model. Mech.* 8(6):591–602
51. Pan H, Yan BS, Rojas M, Shebzukhov YV, Zhou H, et al. 2005. *Ipr1* gene mediates innate immunity to tuberculosis. *Nature* 434(7034):767–72
52. Ji DX, Yamashiro LH, Chen KJ, Mukaida N, Kramnik I, et al. 2019. Type I interferon-driven susceptibility to *Mycobacterium tuberculosis* is mediated by IL-1Ra. *Nat. Microbiol.* 4(12):2128–35
53. Chesler EJ, Miller DR, Branstetter LR, Galloway LD, Jackson BL, et al. 2008. The Collaborative Cross at Oak Ridge National Laboratory: developing a powerful resource for systems genetics. *Mamm. Genome* 19:382–89
54. Churchill GA, Gatti DM, Munger SC, Svenson KL. 2012. The diversity outbred mouse population. *Mamm. Genome* 23(9–10):713–18
55. Ahmed M, Thirunavukkarasu S, Rosa BA, Thomas KA, Das S, et al. 2020. Immune correlates of tuberculosis disease and risk translate across species. *Sci. Transl. Med.* 12(528):eaay0233
56. Smith CM, Proulx MK, Olive AJ, Laddy D, Mishra BB, et al. 2016. Tuberculosis susceptibility and vaccine protection are independently controlled by host genotype. *mBio* 7(5):e01516–16
57. Smith CM, Proulx MK, Lai R, Kiritsy MC, Bell TA, et al. 2019. Functionally overlapping variants control tuberculosis susceptibility in collaborative cross mice. *mBio* 10(6):e02791–19
58. Niazi MKK, Dhulekar N, Schmidt D, Major S, Cooper R, et al. 2015. Lung necrosis and neutrophils reflect common pathways of susceptibility to *Mycobacterium tuberculosis* in genetically diverse, immune-competent mice. *Dis. Model. Mech.* 8(9):1141–53

59. Subbian S, Tsenova L, Yang G, O'Brien P, Parsons S, et al. 2011. Chronic pulmonary cavitary tuberculosis in rabbits: a failed host immune response. *Open Biol.* 1(4):110016
60. Clark S, Hall Y, Williams A. 2015. Animal models of tuberculosis: guinea pigs. *Cold Spring Harb. Perspect. Med.* 5(5):a018572
61. Hoff DR, Ryan GJ, Driver ER, Ssemakulu CC, de Groote MA, et al. 2011. Location of intra- and extracellular *M. tuberculosis* populations in lungs of mice and guinea pigs during disease progression and after drug treatment. *PLOS ONE* 6(3):e17550
62. Ruhl CR, Pasko BL, Khan HS, Kindt LM, Stamm CE, et al. 2020. *Mycobacterium tuberculosis* sulfolipid-1 activates nociceptive neurons and induces cough. *Cell* 181(2):293–305.e11
63. Via LE, Weiner DM, Schimel D, Lin PL, Dayao E, et al. 2013. Differential virulence and disease progression following *Mycobacterium tuberculosis* complex infection of the common marmoset (*Callithrix jacchus*). *Infect. Immun.* 81(8):2909–19
64. Peña JC, Ho WZ. 2015. Monkey models of tuberculosis: lessons learned. *Infect. Immun.* 83(3):852–62
65. Cronan MR, Tobin DM. 2014. Fit for consumption: zebrafish as a model for tuberculosis. *Dis. Model. Mech.* 7(7):777–84
66. Liu CH, Liu H, Ge B. 2017. Innate immunity in tuberculosis: host defense versus pathogen evasion. *Cell. Mol. Immunol.* 14(12):963–75
67. Ernst JD. 1998. Macrophage receptors for *Mycobacterium tuberculosis*. *Infect. Immun.* 66(4):1277–81
68. Arbués A, Malaga W, Constant P, Guilhot C, Prandi J, Astarie-Dequeker C. 2016. Trisaccharides of phenolic glycolipids confer advantages to pathogenic mycobacteria through manipulation of host-cell pattern-recognition receptors. *ACS Chem. Biol.* 11(10):2865–75
69. Blanc L, Gilleron M, Prandi J, Song O, Jang M-S, et al. 2017. *Mycobacterium tuberculosis* inhibits human innate immune responses via the production of TLR2 antagonist glycolipids. *PNAS* 114(42):11205–10
70. Armstrong JA, Hart PD. 1975. Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli: reversal of the usual nonfusion pattern and observations on bacterial survival. *J. Exp. Med.* 142(1):1–16
71. Rajaram MVS, Arnett E, Azad AK, Guirado E, Ni B, et al. 2017. *M. tuberculosis*-initiated human mannose receptor signaling regulates macrophage recognition and vesicle trafficking by FcRγ-chain, Grb2, and SHP-1. *Cell Rep.* 21(1):126–40
72. Astarie-Dequeker C, Le Guyader L, Malaga W, Seaphanh F-K, Chalut C, et al. 2009. Phthiocerol dimycocerosates of *M. tuberculosis* participate in macrophage invasion by inducing changes in the organization of plasma membrane lipids. *PLOS Pathog.* 5(2):e1000289
73. Augenstreich J, Haanappel E, Ferré G, Czaplicki G, Jolibois F, et al. 2019. The conical shape of DIM lipids promotes *Mycobacterium tuberculosis* infection of macrophages. *PNAS* 116(51):25649–58
74. Pauwels A-M, Trost M, Beyaert R, Hoffmann E. 2017. Patterns, receptors, and signals: regulation of phagosome maturation. *Trends Immunol.* 38(6):407–22
75. Sun J, Wang X, Lau A, Liao T-YA, Bucci C, Hmama Z. 2010. Mycobacterial nucleoside diphosphate kinase blocks phagosome maturation in murine raw 264.7 macrophages. *PLOS ONE* 5(1):e8769
76. Roberts EA, Chua J, Kyei GB, Deretic V. 2006. Higher order Rab programming in phagolysosome biogenesis. *J. Cell Biol.* 174(7):923–29
77. Vergne I, Chua J, Deretic V. 2003. *Mycobacterium tuberculosis* phagosome maturation arrest: selective targeting of PI3P-dependent membrane trafficking. *Traffic* 4(9):600–6
78. Vergne I, Chua J, Lee H-H, Lucas M, Belisle J, Deretic V. 2005. Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*. *PNAS* 102(11):4033–38
79. Pradhan G, Shrivastva R, Mukhopadhyay S. 2018. Mycobacterial PknG targets the Rab7l1 signaling pathway to inhibit phagosome–lysosome fusion. *J. Immunol.* 201(5):1421–33
80. Wong D, Bach H, Sun J, Hmama Z, Av-Gay Y. 2011. *Mycobacterium tuberculosis* protein tyrosine phosphatase (PtpA) excludes host vacuolar-H^+–ATPase to inhibit phagosome acidification. *PNAS* 108(48):19371–76
81. Grundner C, Cox JS, Alber T. 2008. Protein tyrosine phosphatase PtpA is not required for *Mycobacterium tuberculosis* growth in mice. *FEMS Microbiol. Lett.* 287(2):181–84
82. Buter J, Cheng T-Y, Ghanem M, Grootemaat AE, Raman S, et al. 2019. *Mycobacterium tuberculosis* releases an antacid that remodels phagosomes. *Nat. Chem. Biol.* 15(9):889–99

83. Vandal OH, Pierini LM, Schnappinger D, Nathan CF, Ehrt S. 2008. A membrane protein preserves intrabacterial pH in intraphagosomal *Mycobacterium tuberculosis*. *Nat. Med.* 14(8):849–54
84. Martinez J, Malireddi RKS, Lu Q, Cunha LD, Pelletier S, et al. 2015. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. *Nat. Cell Biol.* 17(7):893–906
85. Köster S, Upadhyay S, Chandra P, Papavinasasundaram K, Yang G, et al. 2017. *Mycobacterium tuberculosis* is protected from NADPH oxidase and LC3-associated phagocytosis by the LCP protein CpsA. *PNAS* 114(41):E8711–20
86. Sun J, Singh V, Lau A, Stokes RW, Obregón-Henao A, et al. 2013. *Mycobacterium tuberculosis* nucleoside diphosphate kinase inactivates small GTPases leading to evasion of innate immunity. *PLOS Pathog.* 9(7):e1003499
87. Miller JL, Velmurugan K, Cowan MJ, Briken V. 2010. The type I NADH dehydrogenase of *Mycobacterium tuberculosis* counters phagosomal NOX2 activity to inhibit TNF-α-mediated host cell apoptosis. *PLOS Pathog.* 6(4):e1000864
88. Conrad WH, Osman MM, Shanahan JK, Chu F, Takaki KK, et al. 2017. Mycobacterial ESX-1 secretion system mediates host cell lysis through bacterium contact-dependent gross membrane disruptions. *PNAS* 114(6):1371–76
89. Augenstreich J, Arbues A, Simeone R, Haanappel E, Wegener A, et al. 2017. ESX-1 and phthiocerol dimycocerosates of *Mycobacterium tuberculosis* act in concert to cause phagosomal rupture and host cell apoptosis. *Cell. Microbiol.* 19(7):e12726
90. Quigley J, Hughitt VK, Velikovsky CA, Mariuzza RA, El-Sayed NM, Briken V. 2017. The cell wall lipid PDIM contributes to phagosomal escape and host cell exit of *Mycobacterium tuberculosis*. *mBio* 8(2):e00148–17
91. Barczak AK, Avraham R, Singh S, Luo SS, Zhang WR, et al. 2017. Systematic, multiparametric analysis of *Mycobacterium tuberculosis* intracellular infection offers insight into coordinated virulence. *PLOS Pathog.* 13(5):e1006363
92. Mittal E, Skowyra ML, Uwase G, Tinaztepe E, Mehra A, et al. 2018. *Mycobacterium tuberculosis* type VII secretion system effectors differentially impact the ESCRT endomembrane damage response. *mBio* 9(6):e01765-18
93. Collins AC, Cai H, Li T, Franco LH, Li XD, et al. 2015. Cyclic GMP-AMP synthase is an innate immune DNA sensor for *Mycobacterium tuberculosis*. *Cell Host Microbe* 17(6):820–28
94. Wassermann R, Gulen MF, Sala C, Perin SG, Lou Y, et al. 2015. *Mycobacterium tuberculosis* differentially activates cGAS- and inflammasome-dependent intracellular immune responses through ESX-1. *Cell Host Microbe* 17(6):799–810
95. Watson RO, Bell SL, MacDuff DA, Kimmey JM, Diner EJ, et al. 2015. The cytosolic sensor cGAS detects *Mycobacterium tuberculosis* DNA to induce type I interferons and activate autophagy. *Cell Host Microbe* 17(6):811–19
96. Watson RO, Manzanillo PS, Cox JS. 2012. Extracellular *M. tuberculosis* DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell* 150(4):803–15
97. Dey B, Dey RJ, Cheung LS, Pokkali S, Guo H, et al. 2015. A bacterial cyclic dinucleotide activates the cytosolic surveillance pathway and mediates innate resistance to tuberculosis. *Nat. Med.* 21(4):401–6
98. Cheng Y, Schorey JS. 2018. *Mycobacterium tuberculosis*-induced IFN-β production requires cytosolic DNA and RNA sensing pathways. *J. Exp. Med.* 215(11):2919–35
99. Pandey AK, Yang Y, Jiang Z, Fortune SM, Coulombe F, et al. 2009. NOD2, RIP2 and IRF5 play a critical role in the type I interferon response to *Mycobacterium tuberculosis*. *PLOS Pathog.* 5(7):e1000500
100. Mishra BB, Moura-Alves P, Sonawane A, Hacohen N, Griffiths G, et al. 2010. *Mycobacterium tuberculosis* protein ESAT-6 is a potent activator of the NLRP3/ASC inflammasome. *Cell. Microbiol.* 12(8):1046–63
101. Saiga H, Kitada S, Shimada Y, Kamiyama N, Okuyama M, et al. 2012. Critical role of AIM2 in *Mycobacterium tuberculosis* infection. *Int. Immunol.* 24(10):637–44
102. Budzik JM, Swaney DL, Jimenez-Morales D, Johnson JR, Garelis NE, et al. 2020. Dynamic post-translational modification profiling of *M. tuberculosis*-infected primary macrophages. *eLife* 9:e51461
103. Manzanillo PS, Ayres JS, Watson RO, Collins AC, Souza G, et al. 2013. The ubiquitin ligase parkin mediates resistance to intracellular pathogens. *Nature* 501(7468):512–16

104. Franco LH, Nair VR, Scharn CR, Xavier RJ, Torrealba JR, et al. 2017. The ubiquitin ligase Smurf1 functions in selective autophagy of *Mycobacterium tuberculosis* and anti-tuberculous host defense. *Cell Host Microbe* 21(1):59–72
105. Chai Q, Wang X, Qiang L, Zhang Y, Ge P, et al. 2019. A *Mycobacterium tuberculosis* surface protein recruits ubiquitin to trigger host xenophagy. *Nat. Commun.* 10(1):1973
106. Shin D-M, Jeon B-Y, Lee H-M, Jin HS, Yuk J-M, et al. 2010. *Mycobacterium tuberculosis* Eis regulates autophagy, inflammation, and cell death through redox-dependent signaling. *PLOS Pathog.* 6(12):e1001230
107. Seto S, Tsujimura K, Koide Y. 2012. Coronin-1a inhibits autophagosome formation around *Mycobacterium tuberculosis*-containing phagosomes and assists mycobacterial survival in macrophages. *Cell. Microbiol.* 14(5):710–27
108. Saini NK, Baena A, Ng TW, Venkataswamy MM, Kennedy SC, et al. 2016. Suppression of autophagy and antigen presentation by *Mycobacterium tuberculosis* PE_PGRS47. *Nat. Microbiol.* 1(9):16133
109. Shui W, Petzold CJ, Redding A, Liu J, Pitcher A, et al. 2011. Organelle membrane proteomics reveals differential influence of mycobacterial lipoglycans on macrophage phagosome maturation and autophagosome accumulation. *J. Proteome Res.* 10(1):339–48
110. Ouimet M, Koster S, Sakowski E, Ramkhelawon B, van Solingen C, et al. 2016. *Mycobacterium tuberculosis* induces the miR-33 locus to reprogram autophagy and host lipid metabolism. *Nat. Immunol.* 17(6):677–86
111. Kimmey JM, Huynh JP, Weiss LA, Park S, Kambal A, et al. 2015. Unique role for ATG5 in neutrophil-mediated immunopathology during *M. tuberculosis* infection. *Nature* 528(7583):565–69
112. Castillo EF, Dekonenko A, Arko-Mensah J, Mandell MA, Dupont N, et al. 2012. Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation. *PNAS* 109(46):E3168–76
113. Manca C, Tsenova L, Freeman S, Barczak AK, Tovey M, et al. 2005. Hypervirulent *M. tuberculosis* W/Beijing strains upregulate type I IFNs and increase expression of negative regulators of the Jak-Stat pathway. *J. Interf. Cytokine Res.* 25(11):694–701
114. Berry MPR, Graham CM, McNab FW, Xu Z, Bloch SAA, et al. 2010. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 466(7309):973–77
115. Dorhoi A, Yeremeev V, Nouailles G, Weiner J, Jörg S, et al. 2014. Type I IFN signaling triggers immunopathology in tuberculosis-susceptible mice by modulating lung phagocyte dynamics. *Eur. J. Immunol.* 44(8):2380–93
116. Kimmey JM, Campbell JA, Weiss LA, Monte KJ, Lenschow DJ, Stallings CL. 2017. The impact of ISGylation during *Mycobacterium tuberculosis* infection in mice. *Microbes Infect.* 19(4–5):249–58
117. Moreira-Teixeira L, Sousa J, McNab FW, Torrado E, Cardoso F, et al. 2016. Type I IFN inhibits alternative macrophage activation during *Mycobacterium tuberculosis* infection and leads to enhanced protection in the absence of IFN-γ signaling. *J. Immunol.* 197(12):4714–26
118. Cooper AM, Pearl JE, Brooks JV, Ehlers S, Orme IM. 2000. Expression of the nitric oxide synthase 2 gene is not essential for early control of *Mycobacterium tuberculosis* in the murine lung. *Infect. Immun.* 68(12):6879–82
119. Chaussabel D, Semnani RT, McDowell MA, Sacks D, Sher A, Nutman TB. 2003. Unique gene expression profiles of human macrophages and dendritic cells to phylogenetically distinct parasites. *Blood* 102(2):672–81
120. Bogunovic D, Byun M, Durfee LA, Abhyankar A, Mansouri D, et al. 2012. Mycobacterial disease and impaired IFN-γ immunity in humans with inherited ISG15 deficiency. *Science* 337(6102):1684–88
121. Zhang X, Bogunovic D, Payelle-Brogard B, Francois-Newton V, Speer SD, et al. 2015. Human intracellular ISG15 prevents interferon-α/β over-amplification and auto-inflammation. *Nature* 517(7532):89–93
122. McNab FW, Ewbank J, Howes A, Moreira-Teixeira L, Martirosyan A, et al. 2014. Type I IFN induces IL-10 production in an IL-27–independent manner and blocks responsiveness to IFN-γ for production of IL-12 and bacterial killing in *Mycobacterium tuberculosis*–infected macrophages. *J. Immunol.* 193(7):3600–12
123. Clifford V, Tebruegge M, Zufferey C, Germano S, Forbes B, et al. 2019. Cytokine biomarkers for the diagnosis of tuberculosis infection and disease in adults in a low prevalence setting. *Tuberculosis* 114:91–102

124. Vinhaes CL, Oliveira-de-Souza D, Silveira-Mattos PS, Nogueira B, Shi R, et al. 2019. Changes in inflammatory protein and lipid mediator profiles persist after antitubercular treatment of pulmonary and extrapulmonary tuberculosis: a prospective cohort study. *Cytokine* 123:154759
125. Moreira-Teixeira L, Redford PS, Stavropoulos E, Ghilardi N, Maynard CL, et al. 2017. T cell-derived IL-10 impairs host resistance to *Mycobacterium tuberculosis* infection. *J. Immunol.* 199(2):613–23
126. Huynh JP, Lin CC, Kimmey JM, Jarjour NN, Schwarzkopf EA, et al. 2018. Bhlhe40 is an essential repressor of IL-10 during *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 215(7):1823–38
127. Mayer-Barber KD, Andrade BB, Barber DL, Hieny S, Feng CG, et al. 2011. Innate and adaptive interferons suppress IL-1α and IL-1β production by distinct pulmonary myeloid subsets during *Mycobacterium tuberculosis* infection. *Immunity* 35:1023–34
128. Netea MG, van de Veerdonk FL, van der Meer JWM, Dinarello CA, Joosten LAB. 2015. Inflammasome-independent regulation of IL-1-family cytokines. *Annu. Rev. Immunol.* 33:49–77
129. Bohrer AC, Tocheny C, Assmann M, Ganusov VV, Mayer-Barber KD. 2018. IL-1R1 mediates host resistance to *Mycobacterium tuberculosis* by *trans*-protection of infected cells. *J. Immunol.* 201(6):1645–50
130. Mayer-Barber KD, Andrade BB, Oland SD, Amaral EP, Barber DL, et al. 2014. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. *Nature* 511(7507):99–103
131. Chen M, Divangahi M, Gan H, Shin DSJ, Hong S, et al. 2008. Lipid mediators in innate immunity against tuberculosis: opposing roles of PGE_2 and LXA_4 in the induction of macrophage death. *J. Exp. Med.* 205(12):2791–801
132. Bafica A, Scanga CA, Serhan C, Machado F, White S, et al. 2005. Host control of *Mycobacterium tuberculosis* is regulated by 5-lipoxygenase-dependent lipoxin production. *J. Clin. Investig.* 115(6):1601–6
133. Mishra BB, Lovewell RR, Olive AJ, Zhang G, Wang W, et al. 2017. Nitric oxide prevents a pathogen-permissive granulocytic inflammation during tuberculosis. *Nat. Microbiol.* 2:17072
134. Blomgran R, Desvignes L, Briken V, Ernst JD. 2012. *Mycobacterium tuberculosis* inhibits neutrophil apoptosis, leading to delayed activation of naive CD4 T cells. *Cell Host Microbe* 11(1):81–90
135. Martin CJ, Booty MG, Rosebrock TR, Nunes-Alves C, Desjardins DM, et al. 2012. Efferocytosis is an innate antibacterial mechanism. *Cell Host Microbe* 12(3):289–300
136. Lerner TR, Borel S, Greenwood DJ, Repnik U, Russell MRG, et al. 2017. *Mycobacterium tuberculosis* replicates within necrotic human macrophages. *J. Cell Biol.* 216(3):583–94
137. Dallenga T, Repnik U, Corleis B, Eich J, Reimer R, et al. 2017. *M. tuberculosis*-induced necrosis of infected neutrophils promotes bacterial growth following phagocytosis by macrophages. *Cell Host Microbe* 22(4):519–30.e3
138. Hinchey J, Lee S, Jeon BY, Basaraba RJ, Venkataswamy MM, et al. 2007. Enhanced priming of adaptive immunity by a proapoptotic mutant of *Mycobacterium tuberculosis*. *J. Clin. Investig.* 117(8):2279–88
139. Kim KH, An DR, Song J, Yoon JY, Kim HS, et al. 2012. *Mycobacterium tuberculosis* Eis protein initiates suppression of host immune responses by acetylation of DUSP16/MKP-7. *PNAS* 109(20):7729–34
140. Schaaf K, Smith SR, Duverger A, Wagner F, Wolschendorf F, et al. 2017. *Mycobacterium tuberculosis* exploits the PPM1A signaling pathway to block host macrophage apoptosis. *Sci. Rep.* 7:42101
141. Amaral EP, Costa DL, Namasivayam S, Riteau N, Kamenyeva O, et al. 2019. A major role for ferroptosis in *Mycobacterium tuberculosis*-induced cell death and tissue necrosis. *J. Exp. Med.* 216(3):556–70
142. Wong K-W, Jacobs WR Jr. 2011. Critical role for NLRP3 in necrotic death triggered by *Mycobacterium tuberculosis*. *Cell. Microbiol.* 13(9):1371–84
143. Welin A, Eklund D, Stendahl O, Lerm M. 2011. Human macrophages infected with a high burden of ESAT-6-expressing *M. tuberculosis* undergo caspase-1- and cathepsin B-independent necrosis. *PLOS ONE* 6(5):e20302
144. Pajuelo D, Gonzalez-Juarbe N, Tak U, Sun J, Orihuela CJ, Niederweis M. 2018. NAD^+ depletion triggers macrophage necroptosis, a cell death pathway exploited by *Mycobacterium tuberculosis*. *Cell Rep.* 24(2):429–40
145. Zhao X, Khan N, Gan H, Tzelepis F, Nishimura T, et al. 2017. Bcl-x_L mediates RIPK3-dependent necrosis in *M. tuberculosis*-infected macrophages. *Mucosal Immunol.* 10(6):1553–68
146. Gleeson LE, Sheedy FJ, Palsson-McDermott EM, Triglia D, O'Leary SM, et al. 2016. *Mycobacterium tuberculosis* induces aerobic glycolysis in human alveolar macrophages that is required for control of intracellular bacillary replication. *J. Immunol.* 196(6):2444–49

147. Braverman J, Sogi KM, Benjamin D, Nomura DK, Stanley SA. 2016. HIF-1α is an essential mediator of IFN-γ-dependent immunity to *Mycobacterium tuberculosis*. *J. Immunol.* 197(4):1287–97
148. El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson RW, et al. 2008. Toll-like receptor-induced arginase 1 in macrophages thwarts effective immunity against intracellular pathogens. *Nat. Immunol.* 9(12):1399–406
149. Huang L, Nazarova EV, Tan S, Liu Y, Russell DG. 2018. Growth of *Mycobacterium tuberculosis* in vivo segregates with host macrophage metabolism and ontogeny. *J. Exp. Med.* 215(4):1135–52
150. Pisu D, Huang L, Grenier JK, Russell DG. 2020. Dual RNA-seq of Mtb-infected macrophages in vivo reveals ontologically distinct host-pathogen interactions. *Cell Rep.* 30(2):335–50.e4
151. Nair S, Huynh JP, Lampropoulou V, Loginicheva E, Esaulova E, et al. 2018. *Irg1* expression in myeloid cells prevents immunopathology during *M. tuberculosis* infection. *J. Exp. Med.* 215(4):1035–45
152. Gautam US, Foreman TW, Bucsan AN, Veatch AV, Alvarez X, et al. 2018. In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of *Mycobacterium tuberculosis*. *PNAS* 115(1):E62–71
153. Nandi B, Behar SM. 2011. Regulation of neutrophils by interferon-γ limits lung inflammation during tuberculosis infection. *J. Exp. Med.* 208(11):2251–62
154. Braverman J, Stanley SA. 2017. Nitric oxide modulates macrophage responses to *Mycobacterium tuberculosis* infection through activation of HIF-1α and repression of NF-κB. *J. Immunol.* 199(5):1805–16
155. Mishra BB, Rathinam VAK, Martens GW, Martinot AJ, Kornfeld H, et al. 2013. Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1β. *Nat. Immunol.* 14(1):52–60
156. Davis AS, Vergne I, Master SS, Kyei GB, Chua J, Deretic V. 2007. Mechanism of inducible nitric oxide synthase exclusion from mycobacterial phagosomes. *PLOS Pathog.* 3(12):e186
157. Dorhoi A, Iannaccone M, Farinacci M, Faé KC, Schreiber J, et al. 2013. MicroRNA-223 controls susceptibility to tuberculosis by regulating lung neutrophil recruitment. *J. Clin. Investig.* 123(11):4836–48
158. Chinta KC, Rahman MA, Saini V, Glasgow JN, Reddy VP, et al. 2018. Microanatomic distribution of myeloid heme oxygenase-1 protects against free radical-mediated immunopathology in human tuberculosis. *Cell Rep.* 25(7):1938–52.e5
159. Eum SY, Kong JH, Hong MS, Lee YJ, Kim JH, et al. 2010. Neutrophils are the predominant infected phagocytic cells in the airways of patients with active pulmonary TB. *Chest* 137(1):122–28
160. La Manna MP, Orlando V, Paraboschi EM, Tamburini B, Di Carlo P, et al. 2019. *Mycobacterium tuberculosis* drives expansion of low-density neutrophils equipped with regulatory activities. *Front. Immunol.* 10:2761
161. Scanga CA, Mohan VP, Yu K, Joseph H, Tanaka K, et al. 2000. Depletion of $Cd4^+$ T cells causes reactivation of murine persistent tuberculosis despite continued expression of interferon γ and nitric oxide synthase 2. *J. Exp. Med.* 192(3):347–58
162. Ahmed A, Rakshit S, Vyakarnam A. 2016. HIV-TB co-infection: mechanisms that drive reactivation of *Mycobacterium tuberculosis* in HIV infection. *Oral Dis.* 22:53–60
163. Serbina NV, Lazarevic V, Flynn JL. 2001. $CD4^+$ T cells are required for the development of cytotoxic $CD8^+$ T cells during *Mycobacterium tuberculosis* infection. *J. Immunol.* 167(12):6991–7000
164. Green AM, DiFazio R, Flynn JL. 2013. IFN-γ from CD4 T cells is essential for host survival and enhances CD8 T cell function during *Mycobacterium tuberculosis* infection. *J. Immunol.* 190(1):270–77
165. Jayaraman P, Sada-Ovalle I, Beladi S, Anderson AC, Dardalhon V, et al. 2010. Tim3 binding to galectin-9 stimulates antimicrobial immunity. *J. Exp. Med.* 207(11):2343–54
166. Gallegos AM, van Heijst JWJ, Samstein M, Su X, Pamer EG, Glickman MS. 2011. A gamma interferon independent mechanism of CD4 T cell mediated control of *M. tuberculosis* infection in vivo. *PLOS Pathog.* 7(5):e1002052
167. Sakai S, Kauffman KD, Sallin MA, Sharpe AH, Young HA, et al. 2016. CD4 T cell-derived IFN-γ plays a minimal role in control of pulmonary *Mycobacterium tuberculosis* infection and must be actively repressed by PD-1 to prevent lethal disease. *PLOS Pathog.* 12(5):e1005667
168. Elias D, Wolday D, Akuffo H, Petros B, Bronner U, Britton S. 2001. Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guérin (BCG) vaccination. *Clin. Exp. Immunol.* 123(2):219–25

169. Ashenafi S, Aderaye G, Bekele A, Zewdie M, Aseffa G, et al. 2014. Progression of clinical tuberculosis is associated with a Th2 immune response signature in combination with elevated levels of SOCS3. *Clin. Immunol.* 151(2):84–99
170. Nikitina IY, Panteleev AV, Kosmiadi GA, Serdyuk YV, Nenasheva TA, et al. 2018. Th1, Th17, and Th1Th17 lymphocytes during tuberculosis: Th1 lymphocytes predominate and appear as low-differentiated $CXCR3^{+}CCR6^{+}$ cells in the blood and highly differentiated $CXCR3^{+/-}CCR6^{-}$ cells in the lungs. *J. Immunol.* 200(6):2090–103
171. Gopal R, Monin L, Slight S, Uche U, Blanchard E, et al. 2014. Unexpected role for IL-17 in protective immunity against hypervirulent *Mycobacterium tuberculosis* HN878 infection. *PLOS Pathog.* 10(5):e1004099
172. Woodworth JS, Wu Y, Behar SM. 2008. *Mycobacterium tuberculosis*-specific $CD8^{+}$ T cells require perforin to kill target cells and provide protection in vivo. *J. Immunol.* 181(12):8595–603
173. Behar SM, Dascher CC, Grusby MJ, Wang C, Brenner MB, et al. 1999. Susceptibility of mice deficient in CD1D or TAP1 to infection with *Mycobacterium tuberculosis*. *J. Exp. Med.* 189(12):1973–80
174. van Pinxteren LAH, Cassidy JP, Smedegaard BHC, Agger EM, Andersen P. 2000. Control of latent *Mycobacterium tuberculosis* infection is dependent on CD8 T cells. *Eur. J. Immunol.* 30(12):3689–98
175. Nunes-Alves C, Booty MG, Carpenter SM, Rothchild AC, Martin CJ, et al. 2015. Human and murine clonal CD8+ T cell expansions arise during tuberculosis because of TCR selection. *PLOS Pathog.* 11(5):e1004849
176. Kursar M, Koch M, Mittrücker H-W, Nouailles G, Bonhagen K, et al. 2007. Regulatory T cells prevent efficient clearance of *Mycobacterium tuberculosis*. *J. Immunol.* 178(5):2661–65
177. Shafiani S, Tucker-Heard G, Kariyone A, Takatsu K, Urdahl KB. 2010. Pathogen-specific regulatory T cells delay the arrival of effector T cells in the lung during early tuberculosis. *J. Exp. Med.* 207(7):1409–20
178. Marin ND, París SC, Vélez VM, Rojas CA, Rojas M, García LF. 2010. Regulatory T cell frequency and modulation of IFN-gamma and IL-17 in active and latent tuberculosis. *Tuberculosis* 90(4):252–61
179. Day CL, Abrahams DA, Bunjun R, Stone L, de Kock M, et al. 2018. PD-1 expression on *Mycobacterium tuberculosis*-specific CD4 T cells is associated with bacterial load in human tuberculosis. *Front. Immunol.* 9:1995
180. Barber DL, Mayer-Barber KD, Feng CG, Sharpe AH, Sher A. 2011. CD4 T cells promote rather than control tuberculosis in the absence of PD-1-mediated inhibition. *J. Immunol.* 186(3):1598–607
181. Anastasopoulou A, Ziogas DC, Samarkos M, Kirkwood JM, Gogas H. 2019. Reactivation of tuberculosis in cancer patients following administration of immune checkpoint inhibitors: current evidence and clinical practice recommendations. *J. Immunother. Cancer* 7(1):239
182. Tzelepis F, Blagih J, Khan N, Gillard J, Mendonca L, et al. 2018. Mitochondrial cyclophilin D regulates T cell metabolic responses and disease tolerance to tuberculosis. *Sci. Immunol.* 3(23):eaar4135
183. Chancellor A, White A, Tocheva AS, Fenn JR, Dennis M, et al. 2017. Quantitative and qualitative iNKT repertoire associations with disease susceptibility and outcome in macaque tuberculosis infection. *Tuberculosis* 105:86–95
184. Sada-Ovalle I, Chiba A, Gonzales A, Brenner MB, Behar SM. 2008. Innate invariant NKT cells recognize *Mycobacterium tuberculosis*-infected macrophages, produce interferon-γ, and kill intracellular bacteria. *PLOS Pathog.* 4(12):e1000239
185. Rothchild AC, Jayaraman P, Nunes-Alves C, Behar SM. 2014. iNKT cell production of GM-CSF controls *Mycobacterium tuberculosis*. *PLOS Pathog.* 10(1):e1003805
186. Sugawara I, Yamada H, Mizuno S, Li CY, Nakayama T, Taniguchi M. 2002. Mycobacterial infection in natural killer T cell knockout mice. *Tuberculosis* 82(2):97–104
187. Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM, et al. 2010. Human mucosal associated invariant T cells detect bacterially infected cells. *PLOS Biol.* 8(6):e1000407
188. Wong EB, Gold MC, Meermeier EW, Xulu BZ, Khuzwayo S, et al. 2019. $TRAV1\text{-}2^{+}$ $CD8^{+}$ T-cells including oligoconal expansions of MAIT cells are enriched in the airways in human tuberculosis. *Commun. Biol.* 2(1):203
189. Chen ZW. 2016. Protective immune responses of major Vγ2Vδ2 T-cell subset in *M. tuberculosis* infection. *Curr. Opin. Immunol.* 42:105–12

190. Shen H, Wang Y, Chen CY, Frencher J, Huang D, et al. 2015. Th17-related cytokines contribute to recall-like expansion/effector function of HMBPP-specific Vγ2Vδ2 T cells after *Mycobacterium tuberculosis* infection or vaccination. *Eur. J. Immunol.* 45(2):442–51
191. Qaqish A, Huang D, Chen CY, Zhang Z, Wang R, et al. 2017. Adoptive transfer of phosphoantigen-specific γδ T cell subset attenuates *Mycobacterium tuberculosis* infection in nonhuman primates. *J. Immunol.* 198(12):4753–63
192. Junqueira-Kipnis AP, Kipnis A, Jamieson A, Juarrero MG, Diefenbach A, et al. 2003. NK cells respond to pulmonary infection with *Mycobacterium tuberculosis*, but play a minimal role in protection. *J. Immunol.* 171(11):6039–45
193. Yoneda T, Ellner JJ. 1998. $CD4^+$ T cell and natural killer cell-dependent killing of *Mycobacterium tuberculosis* by human monocytes. *Am. J. Respir. Crit. Care Med.* 158(2):395–403
194. Brill KJ, Li Q, Larkin R, Canaday DH, Kaplan DR, et al. 2001. Human natural killer cells mediate killing of intracellular *Mycobacterium tuberculosis* H37Rv via granule-independent mechanisms. *Infect. Immun.* 69(3):1755–65
195. Zhang R, Zheng X, Li B, Wei H, Tian Z. 2006. Human NK cells positively regulate γδ T cells in response to *Mycobacterium tuberculosis*. *J. Immunol*. 176(4):2610–16
196. Ardain A, Domingo-Gonzalez R, Das S, Kazer SW, Howard NC, et al. 2019. Group 3 innate lymphoid cells mediate early protective immunity against tuberculosis. *Nature* 570:528–32
197. Ulrichs T, Kosmiadi GA, Trusov V, Jörg S, Pradl L, et al. 2004. Human tuberculous granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defence in the lung. *J. Pathol.* 204(2):217–28
198. Slight SR, Rangel-Moreno J, Gopal R, Lin Y, Junecko BAF, et al. 2013. $CXCR5^+$ T helper cells mediate protective immunity against tuberculosis. *J. Clin. Investig.* 123(2):712–26
199. Phuah J, Wong EA, Gideon HP, Maiello P, Coleman MT, et al. 2016. Effects of B cell depletion on early *Mycobacterium tuberculosis* infection in cynomolgus macaques. *Infect. Immun.* 84(5):1301–11
200. Lu LL, Chung AW, Rosebrock TR, Ghebremichael M, Yu WH, et al. 2016. A functional role for antibodies in tuberculosis. *Cell* 167(2):433–43.e14
201. Lu LL, Smith MT, Yu KKQ, Luedemann C, Suscovich TJ, et al. 2019. IFN-γ-independent immune markers of *Mycobacterium tuberculosis* exposure. *Nat. Med.* 25(6):977–87
202. Li H, Wang XX, Wang B, Fu L, Liu G, et al. 2017. Latently and uninfected healthcare workers exposed to TB make protective antibodies against *Mycobacterium tuberculosis*. *PNAS* 114(19):5023–28
203. Olivares N, Marquina B, Mata-Espinoza D, Zatarain-Barron ZL, Pinzón CE, et al. 2013. The protective effect of immunoglobulin in murine tuberculosis is dependent on IgG glycosylation. *Pathog. Dis.* 69(3):176–83
204. Maglione PJ, Xu J, Casadevall A, Chan J. 2008. Fcγ receptors regulate immune activation and susceptibility during *Mycobacterium tuberculosis* infection. *J. Immunol.* 180(5):3329–38
205. Hamasur B, Haile M, Pawlowski A, Schröder U, Källenius G, Svenson SB. 2004. A mycobacterial lipoarabinomannan specific monoclonal antibody and its $F(ab')_2$ fragment prolong survival of mice infected with *Mycobacterium tuberculosis*. *Clin. Exp. Immunol.* 138(1):30–38
206. Guirado E, Amat I, Gil O, Díaz J, Arcos V, et al. 2006. Passive serum therapy with polyclonal antibodies against *Mycobacterium tuberculosis* protects against post-chemotherapy relapse of tuberculosis infection in SCID mice. *Microbes Infect.* 8(5):1252–59
207. Foreman TW, Mehra S, LoBato DN, Malek A, Alvarez X, et al. 2016. $CD4^+$ T-cell–independent mechanisms suppress reactivation of latent tuberculosis in a macaque model of HIV coinfection. *PNAS* 113(38):E5636–44
208. Amelio P, Portevin D, Hella J, Reither K, Kamwela L, et al. 2018. HIV infection functionally impairs *Mycobacterium tuberculosis*-specific CD4 and CD8 T-cell responses. *J. Virol.* 93(5):e01728-18
209. Pathak S, Wentzel-Larsen T, Åsjö B. 2010. Effects of in vitro HIV-1 infection on mycobacterial growth in peripheral blood monocyte-derived macrophages. *Infect. Immun.* 78(9):4022–32
210. Lai RPJ, Nakiwala JK, Meintjes G, Wilkinson RJ. 2013. The immunopathogenesis of the HIV tuberculosis immune reconstitution inflammatory syndrome. *Eur. J. Immunol.* 43(8):1995–2002

211. Seddiki N, Sasson SC, Santner-Nanan B, Munier M, van Bockel D, et al. 2009. Proliferation of weakly suppressive regulatory $CD4^+$ T cells is associated with over-active $CD4^+$ T-cell responses in HIV-positive patients with mycobacterial immune restoration disease. *Eur. J. Immunol.* 39(2):391–403
212. Cheekatla SS, Tripathi D, Venkatasubramanian S, Nathella PK, Paidipally P, et al. 2016. NK-CD11c+ cell crosstalk in diabetes enhances IL-6-mediated inflammation during *Mycobacterium tuberculosis* infection. *PLOS Pathog.* 12(10):e1005972
213. Gomez DI, Twahirwa M, Schlesinger LS, Restrepo BI. 2013. Reduced *Mycobacterium tuberculosis* association with monocytes from diabetes patients that have poor glucose control. *Tuberculosis* 93(2):192–97
214. Martinez N, Ketheesan N, West K, Vallerskog T, Kornfeld H. 2016. Impaired recognition of *Mycobacterium tuberculosis* by alveolar macrophages from diabetic mice. *J. Infect. Dis.* 214(11):1629–37
215. Vrieling F, Wilson L, Rensen PCN, Walzl G, Ottenhoff THM, Joosten SA. 2019. Oxidized low-density lipoprotein (oxLDL) supports *Mycobacterium tuberculosis* survival in macrophages by inducing lysosomal dysfunction. *PLOS Pathog.* 15(4):e1007724
216. Chao WC, Yen CL, Wu YH, Chen SY, Hsieh CY, et al. 2015. Increased resistin may suppress reactive oxygen species production and inflammasome activation in type 2 diabetic patients with pulmonary tuberculosis infection. *Microbes Infect.* 17(3):195–204
217. Raposo-García S, Guerra-Laso JM, García-García S, Juan-García J, López-Fidalgo E, et al. 2017. Immunological response to *Mycobacterium tuberculosis* infection in blood from type 2 diabetes patients. *Immunol. Lett.* 186:41–45
218. Tripathi D, Radhakrishnan RK, Thandi RS, Paidipally P, Devalraju KP, et al. 2019. IL-22 produced by type 3 innate lymphoid cells (ILC3s) reduces the mortality of type 2 diabetes mellitus (T2DM) mice infected with *Mycobacterium tuberculosis*. *PLOS Pathog.* 15(12):e1008140
219. Vallerskog T, Martens GW, Kornfeld H. 2010. Diabetic mice display a delayed adaptive immune response to *Mycobacterium tuberculosis*. *J. Immunol.* 184(11):6275–82
220. Feng Y, Kong Y, Barnes PF, Huang FF, Klucar P, et al. 2011. Exposure to cigarette smoke inhibits the pulmonary T-cell response to influenza virus and *Mycobacterium tuberculosis*. *Infect. Immun.* 79(1):229–37
221. Berg RD, Levitte S, O'Sullivan MP, O'Leary SM, Cambier CJ, et al. 2016. Lysosomal disorders drive susceptibility to tuberculosis by compromising macrophage migration. *Cell* 165(1):139–52
222. O'Leary SM, Coleman MM, Chew WM, Morrow C, McLaughlin AM, et al. 2014. Cigarette smoking impairs human pulmonary immunity to *Mycobacterium tuberculosis*. *Am. J. Respir. Crit. Care Med.* 190(12):1430–36
223. Mason CM, Dobard E, Zhang P, Nelson S. 2004. Alcohol exacerbates murine pulmonary tuberculosis. *Infect. Immun.* 72(5):2556–63
224. Tripathi D, Welch E, Cheekatla SS, Radhakrishnan RK, Venkatasubramanian S, et al. 2018. Alcohol enhances type 1 interferon-α production and mortality in young mice infected with *Mycobacterium tuberculosis*. *PLOS Pathog.* 14(8):e1007174
225. Cegielski JP, McMurray DN. 2004. The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. *Int. J. Tuberc. Lung Dis.* 8(3):286–98
226. Bourke CD, Berkley JA, Prendergast AJ. 2016. Immune dysfunction as a cause and consequence of malnutrition. *Trends Immunol.* 37(6):386–98
227. Vilchèze C, Jacobs WR Jr. 2014. Resistance to isoniazid and ethionamide in *Mycobacterium tuberculosis*: genes, mutations, and causalities. *Microbiol. Spectr.* 2(4):MGM2-0014-2013
228. Lin W, Mandal S, Degen D, Liu Y, Ebright YW, et al. 2017. Structural basis of *Mycobacterium tuberculosis* transcription and transcription inhibition. *Mol. Cell* 66(2):169–79.e8
229. Jagielski T, Bakuła Z, Brzostek A, Minias A, Law Stachowiak R, et al. 2018. Characterization of mutations conferring resistance to rifampin in *Mycobacterium tuberculosis* clinical strains. *Antimicrob. Agents Chemother.* 62(10):e01093-18
230. Lamont EA, Dillon NA, Baughn AD. 2020. The bewildering antitubercular action of pyrazinamide. *Microbiol. Mol. Biol. Rev.* 84(2):e00070-19
231. Mikusova K, Slayden RA, Besra GS, Brennan PJ. 1995. Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. *Antimicrob. Agents Chemother.* 39(11):2484–89
232. Sreevatsan S, Stockbauer KE, Pan X, Kreiswirth BN, Moghazeh SL, et al. 1997. Ethambutol resistance in *Mycobacterium tuberculosis*: critical role of *embB* mutations. *Antimicrob. Agents Chemother.* 41(8):1677–81

233. Zhao L, Sun Q, Liu H, Wu X, Xiao T, et al. 2015. Analysis of embCAB mutations associated with ethambutol resistance in multidrug-resistant *Mycobacterium tuberculosis* isolates from China. *Antimicrob. Agents Chemother.* 59(4):2045–50
234. Plinke C, Cox HS, Zarkua N, Karimovich HA, Braker K, et al. 2010. *embCAB* sequence variation among ethambutol-resistant *Mycobacterium tuberculosis* isolates without *embB306* mutation. *J. Antimicrob. Chemother.* 65(7):1359–67