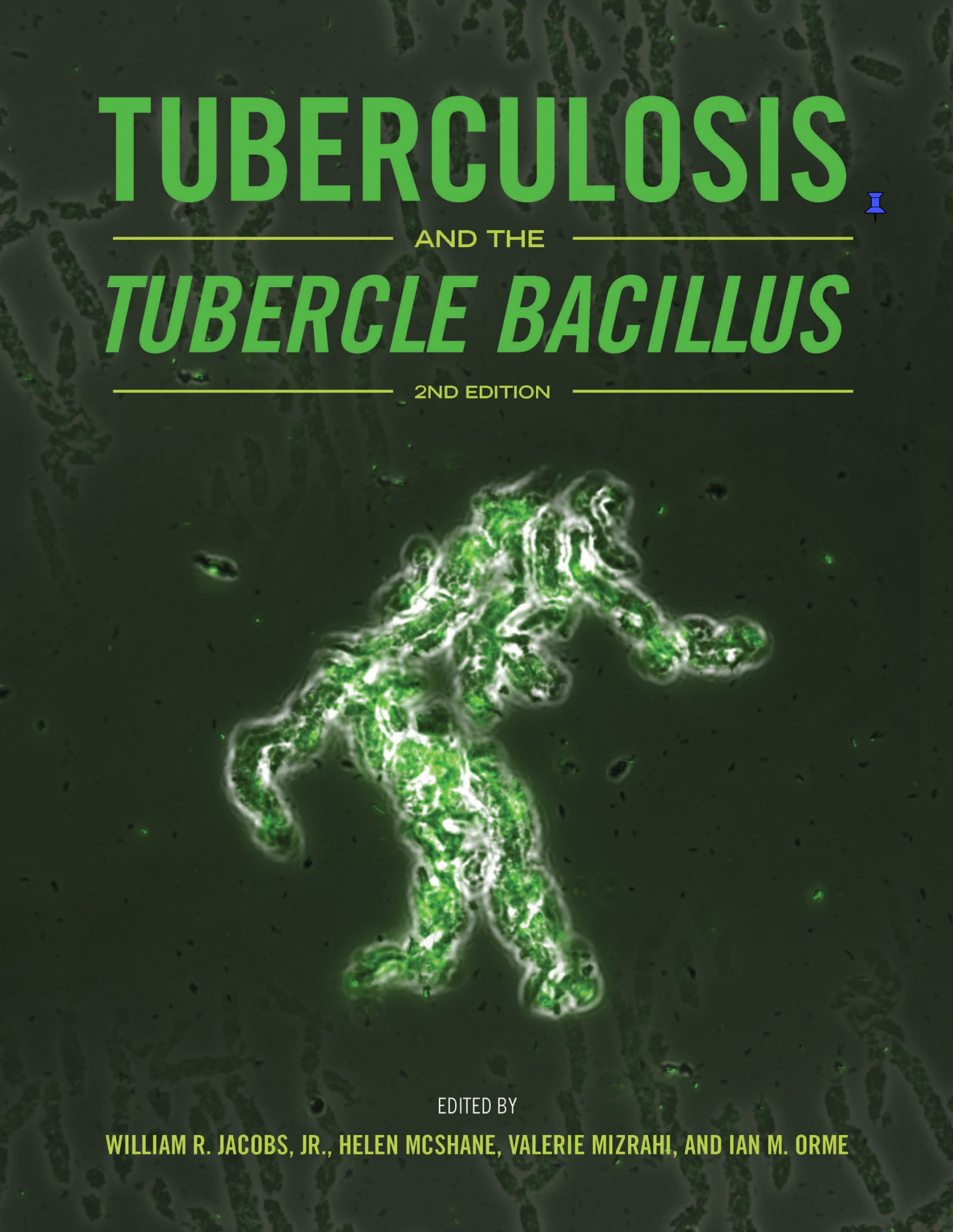
TUBERCULOSIS

AND THE

TUBERCLE BACILLUS

2ND EDITION



EDITED BY

WILLIAM R. JACOBS, JR., HELEN MCSHANE, VALERIE MIZRAHI, AND IAN M. ORME

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Preface: Combating Tuberculosis: Edward Jenner's Revenge

It is the height of irony that the man who discovered the smallpox vaccine, Edward Jenner, lost both his wife and son to tuberculosis (TB). By the time smallpox was essentially eradicated, it is estimated that over 300 million people had died from this disease over the preceding century. Its eventual prevention—by a simple vaccine—clearly illustrates the power of scientific discovery and how its application can affect human health. Hundreds of millions of people have been spared death and suffering from infectious diseases because of the development of vaccines and chemotherapeutic agents in the last 100 years. Millions of lives have been saved with the use of the TB vaccine, BCG, and the development of chemotherapeutic regimens for TB. Depressingly, despite these effective interventions, TB remains one of the most challenging problems of global health, with over 9 million new cases and 1.6 million deaths each year. This crisis has been further compounded by the emergence of the HIV epidemic, as this explosive and deadly combination has dramatically increased the global spread of TB, including increasing numbers of cases of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB.

Historically, mycobacterial disease has long been at the forefront of scientific discovery for infectious diseases. The leprosy bacillus, *Mycobacterium leprae*, the first bacterium to be associated with human disease, was initially visualized by Gerhard Armauer Hansen in 1873. Earlier, Jean Antoine Villemin was the first person to realize that lung tubercles were infectious and not cancerous. By the 1880s, Robert Koch, aware of both of these discoveries, not only observed the tubercle bacilli in tubercles, but developed a growth medium of heated serum to cultivate the tubercle bacillus outside of humans. He went on to repeat the transfer experiment of Villemin and transferred the disease of TB to numerous animal species, establishing the experimental paradigm ("the postulates") of how to prove that an infectious agent is a cause of a disease. Koch's findings led Albert Calmette and Camille Guérin to follow Jenner's approach of developing an attenuated pathogen for use as a vaccine, using the bovine tubercle bacillus to develop the bacille Calmette-Guérin (BCG) vaccine that bears their names and is still used to this day.

It is noteworthy that Paul Ehrlich was sitting in the lecture hall when Robert Koch presented his work in 1882; he later went on to help Koch improve his staining techniques. By observing the selective staining of various cell types, including human cells and different bacteria, Ehrlich also developed the idea of chemotherapy—"magic bullets" that could kill microbial pathogens. He tried for years to develop a chemical that could kill the tubercle bacillus, with little success, though at the same time was far more successful in developing a treatment for syphilis. In the 1930s, his protégé Gerhard Domagk discovered the first sulfonamide to treat bacterial infections such as streptococcus, and as this fledging field expanded, para-amino salicylic acid and isoniazid were discovered to be active against the TB bacillus. Parallel studies by Salman Waksman and Albert Schatz in the 1950s led to the discovery of streptomycin, the first bactericidal drug for the tubercle bacilli.

Despite these many historical advances, the TB bacillus—*Mycobacterium tuberculosis*—has proven to be a formidable adversary against numerous interventions. Nevertheless, despite the arduous challenges of

working with this dangerous pathogen, the field continues to persevere, and our continued success in the pursuit of knowledge would, we suspect, be applauded by Koch, Ehrlich, Calmette, and many others, as we strive to find and apply more effective cures for this dreadful disease. In this spirit, this textbook is a collection of state-of-the-art research aimed at understanding the TB bacillus, the way it infects its host, the mechanisms by which it persists in the face of host immunity, and current intervention and therapeutic methods. The contributors of this book believe that such continued and dedicated research efforts will eventually lead to better vaccines, better chemotherapies, and ultimately the eradication of TB—Edward Jenner's revenge.

WILLIAM R. JACOBS, JR.
HELEN MCSHANE
VALERIE MIZRAHI
IAN M. ORME

Towards Edward Jenner's Revenge: Developing an Effective Tuberculosis Vaccine

I

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A. BASIC IMMUNOLOGY

Innate Immune Responses to Tuberculosis

1

Jeffrey S. Schorey[1] and Larry S. Schlesinger[2]

INTRODUCTION

Tuberculosis (TB) remains one of the leading causes of death by an infectious agent, accounting for approximately 1.3 million deaths per year (1). Despite its clinical significance, there are still significant gaps in our understanding of *Mycobacterium tuberculosis* pathogenesis and the host mechanisms that limit active disease to approximately 10% of those infected. Nevertheless, we continue to gain insight into the dynamic interplay between pathogen and host, with much of the focus centered on the lung microenvironment because this is the initial and primary site of infection. The lung as the initial "battlefield" provides unique challenges to both the host and pathogen because the host must balance the inflammatory response to limit the damage to lung tissue while inducing a sufficient immune response to control the infection. In contrast, the *M. tuberculosis* organism must avoid or circumvent the initial defensive barriers present within the respiratory tract to gain access to its host cell, the alveolar macrophage (AM). The AM response to infection as well as the reaction of other lung immune and nonimmune cells and noncellular components is critical to determining whether the host will directly eliminate the pathogen or will in concert with the acquired immune system develop a protective granulomatous response. In addition, since bacteria disseminate during the early events in infection, engagement of innate immune components outside of the lung is also critical in shaping the host response. These early host processes which constitute the innate immune system will be the focus of this article.

LUNG

The lung is responsible for mediating gas exchange across a respiratory surface of 130 m^2, which is more than 60 times the surface of the body (2–4) (Fig. 1). On average, this involves inhalation of 14,000 liters of air each day (5) and processing not only of CO_2 and O_2, but also of pollutants, allergens, and microbes from the inhaled air. Efficient gas exchange requires a thin barrier between the air and blood; in some instances this barrier is composed of only one endothelial cell and one epithelial cell and is less than 2 m thick. The lung must maintain this thin barrier while clearing inhaled insults (2–4).

The lung uses multiple defenses to clear contaminants from the air (discussed in more detail below). Briefly, basic instincts such as the coughing and sneezing reflex remove particulates from the lung. Mucus, together with cilia, forms the mucociliary escalator which transfers particles up the trachea for removal. Also, epithelial cells in the airway secrete antimicrobials including lysozyme and antimicrobial peptides such as defensins and cathelicidins (6, 7). These defenses clear particulates $\geq$5 μm. Anything smaller passes through the conducting system with velocity, eventually settling in the alveoli, where alveolar cells become responsible for their clearance.

The main three cell types in the alveolus are type I and type II alveolar epithelial cells (AECs) and AMs. These cells are coated by alveolar fluid and surfactant. External to the alveolus is the alveolar septum, which contains blood vessels, fibroblasts, protein fibers, and

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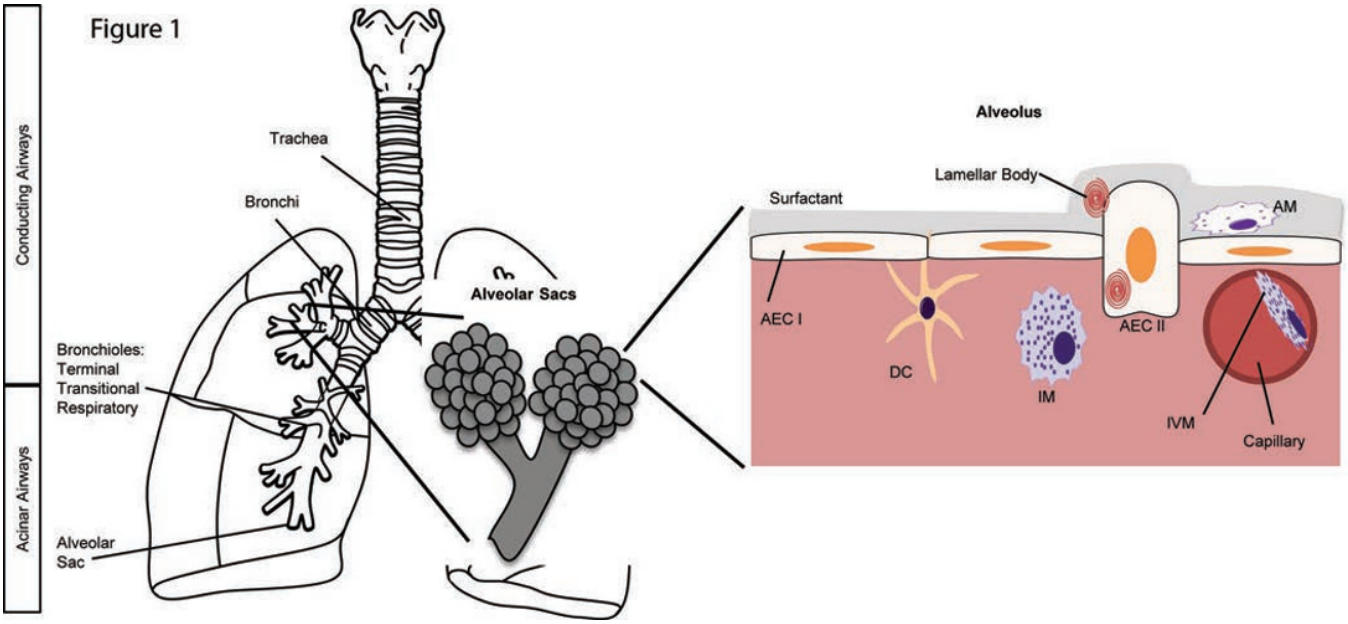

Figure 1 Schematic of the lung and the role of pulmonary innate immune cells during *M. tuberculosis* infection. From left to right: branching of the airways, culminating in the alveolar sacs and the alveolus. Also depicted are the cells in the alveolus. Abbreviations: AEC I and II, type I and II alveolar epithelial cell; AM, alveolar macrophage; DC, dendritic cell; IM, interstitial macrophage; IVM, intravascular macrophage.

pores of Kohn. The fibers are responsible for the structural integrity of the alveolus. The pores of Kohn are holes in the septum that connect alveoli to each other, are filled with surfactant, and provide a passage for cells to migrate between alveoli (2, 8).

Lung Cellular Components

AECs

Although type I AECs constitute only 8% of peripheral lung cells (a third of the cells in the alveolus), they cover ~95% of the alveolar surface. Their thin flat shape, as well as their contact with endothelial cells of the pulmonary capillaries, provides the necessary thin surface for gas exchange to occur (2, 3). The cuboidal type II AECs constitute about 15% of peripheral lung cells, cover 5% of the alveolar surface, and have a smaller surface area than type I AECs—250 μm^2 compared to 5,000 μm^2 (9, 10). Type II AECs contain distinctive lamellar bodies and have apical microvilli. These cells secrete surfactant phospholipids and proteins, as well as lysozyme and antimicrobial peptides in lamellar bodies (7, 9). Following lung damage, type II AECs can serve as precursors to type I cells and self-renew (2, 3, 7). These cells also express HLA class I and II molecules (9), and murine cells can present mycobacterial antigens (11).

Lung macrophages

AMs constitute the majority of cells collected from bronchoalveolar lavage (≥90%). They maintain the alveolar microenvironment, removing debris, dead cells, and microbes. They are long lived, with a half-life of 1 to 8 months (12, 13); about 40% of total AMs turn over each year (14). AMs are thought to originate from peripheral blood monocytes following migration into the lung (15) but may also originate from lung macrophages in response to inflammation (12, 16–20).

AMs must exert tightly regulated pro- and anti-inflammatory actions to control infection without damaging the fragile alveolar environment (14, 21). Thus, they exhibit characteristics of M1 (classically activated) and M2 (alternatively activated) macrophages (22). They express high levels of mannose receptor (MR), scavenger receptor-A, Toll-like receptor 9 (TLR9), and the nuclear receptor peroxisome proliferator-associated receptor gamma (PPARγ), and low levels of TLR2 and the costimulatory molecules CD80 and CD86 (14, 21, 23). PPARγ expression may be important for differentiation of AMs (24), which are highly phagocytic (25) but have a limited oxidative burst relative to neutrophils or peripheral blood mononuclear cells (PBMCs) (25, 26) and are weakly bactericidal (12). AMs are poor antigen presenters (27) and downregulate the abil-

ity of dendritic cells (DCs) to present antigen (28) and suppress lymphocyte activation (29).

The lung contains three types of macrophages, named based on their location: AMs, intravascular macrophages (IVMs), and interstitial macrophages (IMs). IVMs are located in the capillaries on endothelial cells, and IMs are in the interstitial space between alveoli (30, 31). The IVMs and IMs are less well understood than AMs, likely due to the difficulty isolating them. AMs are readily isolated from bronchoalveolar lavage following only a few washes, while IMs are obtained from the bronchoalveolar lavage following many washes (31), and many animals such as mice and humans (in contrast to pigs and horses, for example) may not constitutively produce IVMs (30). In rhesus macaques IMs were shown to have a higher turnover rate and be shorter lived than AMs (32). IMs are thought to regulate tissue fibrosis, inflammation, and antigen presentation (33) and be more proinflammatory than AMs (32). IVMs are phagocytic and may clear erythrocytes and fibrin from the blood (30).

DCs

A few DCs are located in the alveoli interstitial space, but most are in the conducting airways (2, 34). In the alveoli, they sit below the AECs and extend membrane protrusions to sample the inner surface of the airway lumen (35). Following antigen processing and presentation, DCs migrate to local lymph nodes (36) and inducible bronchus-associated lymphoid tissue that forms in response to infection or inflammation (37, 38). There are few lymphatic vessels around the alveoli, so alveolar DCs must migrate through the interstitium to access sites of lymphatic drainage (2). Other immune cells, including T and B cells, are found in low amounts in the interstitium.

Mucosal associated invariant T (MAIT) cells

Major histocompatibility complex (MHC)-related protein 1-restricted MAIT cells are unconventional CD8 T cells (39) and are present in the lung airways. MAIT cells detect antigen-presenting cells infected with fungi and bacteria, including *M. tuberculosis*. Following stimulation, MAIT cells produce gamma interferon (IFN-γ) and tumor necrosis factor (TNF) and lyse infected cells (40–42). They are important *in vivo* during mycobacterial infection. MHC-related protein 1 knockout mice show elevated bacillus Calmette-Guérin (BCG) growth compared to wild-type mice (43). Individuals with active TB have reduced levels of MAIT cells in the peripheral blood and enhanced levels in the lung compared with healthy individuals (40).

Neutrophils

Although under conditions of health lung alveoli contain predominantly macrophages, rapid recruitment of neutrophils is well known to be a key determinant of the innate immune response to bacterial pathogens (44). Neutrophils possess potent extracellular and intracellular killing mechanisms. However, severe bacterial lung infections are caused by excessive neutrophil-mediated inflammation. Neutrophils sense bacteria and/or their components and migrate across epithelia along a chemotactic gradient. Neutrophil sequestration is an essential antibacterial defense mechanism in the lung, which involves multiple steps, including activation of transcription factors, production of chemokines, upregulation of cell adhesion molecules, and enhancement of cell-cell interactions.

Mucus and Surfactant

Mucins are the main glycoproteins in mucus and are either tethered to epithelial cells or secreted. They are produced by submucosal glands and goblet cells, club cells, and alveolar cells in the conducting and peripheral airways. They bind particles and microbes to prevent their adherence to host cells and thus mediate clearance via the mucociliary escalator (7).

Pulmonary surfactant is produced by type II AECs in the alveoli and is a complex mixture of lipids and proteins that bathe cells in the alveolus and reduce surface tension to prevent alveolar collapse during expiration. Dipalmitoyl phosphatidylcholine is the most abundant phospholipid in surfactant (45), but surfactant also contains surfactant proteins (SPs) SP-A, SP-B, SP-C, and SP-D (46, 47). In general, SP-B and SP-C maintain stability of the surfactant lipids, while SP-A and SP-D are immunomodulators (7, 48). SP-A enhances apoptotic cell clearance by macrophages and regulates MR activity, the oxidative burst, and negative regulators of inflammation (49–54); SP-A also enhances macrophage phagocytosis of *M. tuberculosis* (55–58). In contrast, SP-D agglutinates *M. tuberculosis*, which decreases macrophage phagocytosis. However, the bacteria that are still phagocytosed show enhanced phagosome lysosome (PL) fusion and killing (59–61).

Soluble Components in the Surfactant Hypophase

Below the surfactant lipid monolayer is an aqueous hyphase of alveolar lining fluid (ALF) that contains substances with intrinsic antimicrobial properties which contribute to the innate immune response to pathogens within this environment (62). These include complement, antibody, defensins, lysozyme, phospholipases,

protease inhibitors, and homeostatic hydrolases that can affect the *M. tuberculosis* cell wall and host responses (63). ALF is generated, secreted, and recycled by AECs and is essential for maintaining lung function (64). Components of the complement system are produced by AECs (65) and macrophages in the lung (66). The complement system is active in the alveolar space and plays an important role in the microbe-macrophage encounter (67–71). TB is increased in the elderly, and recent studies show how the pulmonary environment, particularly ALF, in old age can potentially modify mucosal immune responses, including those in response to *M. tuberculosis* (72).

M. TUBERCULOSIS INTERACTION WITH THE LUNG

Initial Interactions Following Inhalation

Human ALF contains hydrolases that alter the *M. tuberculosis* cell wall, reducing exposure of mannose-capped lipoarabinomannan (ManLAM) and trehalose 6,6´-dimycolate (TDM; cord factor). ALF treatment of *M. tuberculosis* reduced its association with and intracellular replication in human macrophages and led to increased TNF-α release by macrophages (73). Thus, initial exposure to surfactant may alter the *M. tuberculosis* cell wall before interaction with AMs or AECs and affect subsequent interactions with the host, perhaps by altering the receptor primarily engaged by the bacteria.

Interactions with the Macrophage

Phagocytic receptors

The MR (CD206; MRC1) is highly expressed by AMs and subsets of DCs but not by monocytes (74–78) (Fig. 2). It is the dominant C-type lectin on human AMs and monocyte-derived macrophages, and it recognizes endogenous N-linked glycoproteins (79, 80) and mannose-containing pathogen-associated molecular patterns (PAMPs) (81) via its carbohydrate recognition domains. The MR discriminates between mannose-containing PAMPs based on the degree and nature of mannan motifs. It binds to the mannose caps of ManLAM (82, 83) and the higher-order phosphatidylinositol mannosides (PIMs) that are more mannosylated and found in greater amounts on pathogenic mycobacteria (84), thus differentiating among *M. tuberculosis* strains (85, 86). *M. tuberculosis* is thought to use molecular mimicry to bind the MR and mediate favorable entry into macrophages, and usage of the MR may be a marker of host adaptation (83). Binding to the MR mediates phagocytosis of *M. tuberculosis* and leads to decreased PL fusion (84, 87, 88), acidification (89, 90), and oxidative burst (91), as well as release of anti-inflammatory cytokines (92). MR engagement also leads to increased PPARγ activity, which allows for enhanced survival of *M. tuberculosis* in the macrophage, which is discussed in more detail below (23). The MR can facilitate presentation of lipids and ManLAM and serves as a prototypic pattern recognition receptor (PRR) linking innate and adaptive immunity, which has been exploited to deliver DNA vaccines to antigen-presenting cells (22) and is being used to modulate the immune system for therapeutic and vaccine purposes (93). The MR may also contribute to chronic stages of *M. tuberculosis* infection by mediating homotypic cellular adhesion and giant cell formation (94), which are characteristic of TB granulomas (95, 96).

DC-specific intercellular adhesion molecule 3 grabbing nonintegrin (DC-SIGN) is expressed by DCs and subsets of macrophages (77, 97) (Fig. 2). Its expression by human macrophages is generally low but can be induced following *M. tuberculosis* infection (98) or other stimulation (99). DC-SIGN recognizes mannosylated glycoconjugates such as *M. tuberculosis* ManLAM and PIMs (84, 100, 101). DC-SIGN activation during *M. tuberculosis* infection leads to PL fusion (contrary to the MR) and impedes DC maturation (100, 102).

Macrophage-inducible C-type lectin (Mincle; Clec4e, Clecsf9) is expressed on leukocytes at low levels before activation but is highly expressed on mouse macrophages following stimulation (103) (Fig. 2). Mincle recognizes damaged cells and fungi (104, 105). It also recognizes *M. tuberculosis* TDM, resulting in enhanced inflammatory cytokine production and granuloma formation (106, 107), but is not required for control of *M. tuberculosis* infection in mice (108).

Dectin-1 is a nonclassical C-type lectin that recognizes β-glucans (109) (Fig. 2). It is highly expressed on DCs, macrophages, monocytes, neutrophils, and a subset of T cells (110). Dectin-1 activation in conjunction with TLR4 during *M. tuberculosis* infection induces an interleukin (IL)-17A response (111). Similar to the MR, Dectin-1 differentiates between mycobacterium strains; activation by nonpathogenic (*Mycobacterium smegmatis*, *Mycobacterium phlei*) and attenuated (*Mycobacterium bovis* BCG, *M. tuberculosis* $H_{37}R_a$) mycobacteria, but not virulent *M. tuberculosis* $H_{37}R_v$, enhances TNF-α, IL-6, and RANTES production by macrophages (112, 113). Dectin-1 activation inhibits replication of BCG, but not virulent *M. tuberculosis*, in human macrophages (114). Dectin-2 recognizes mannose-

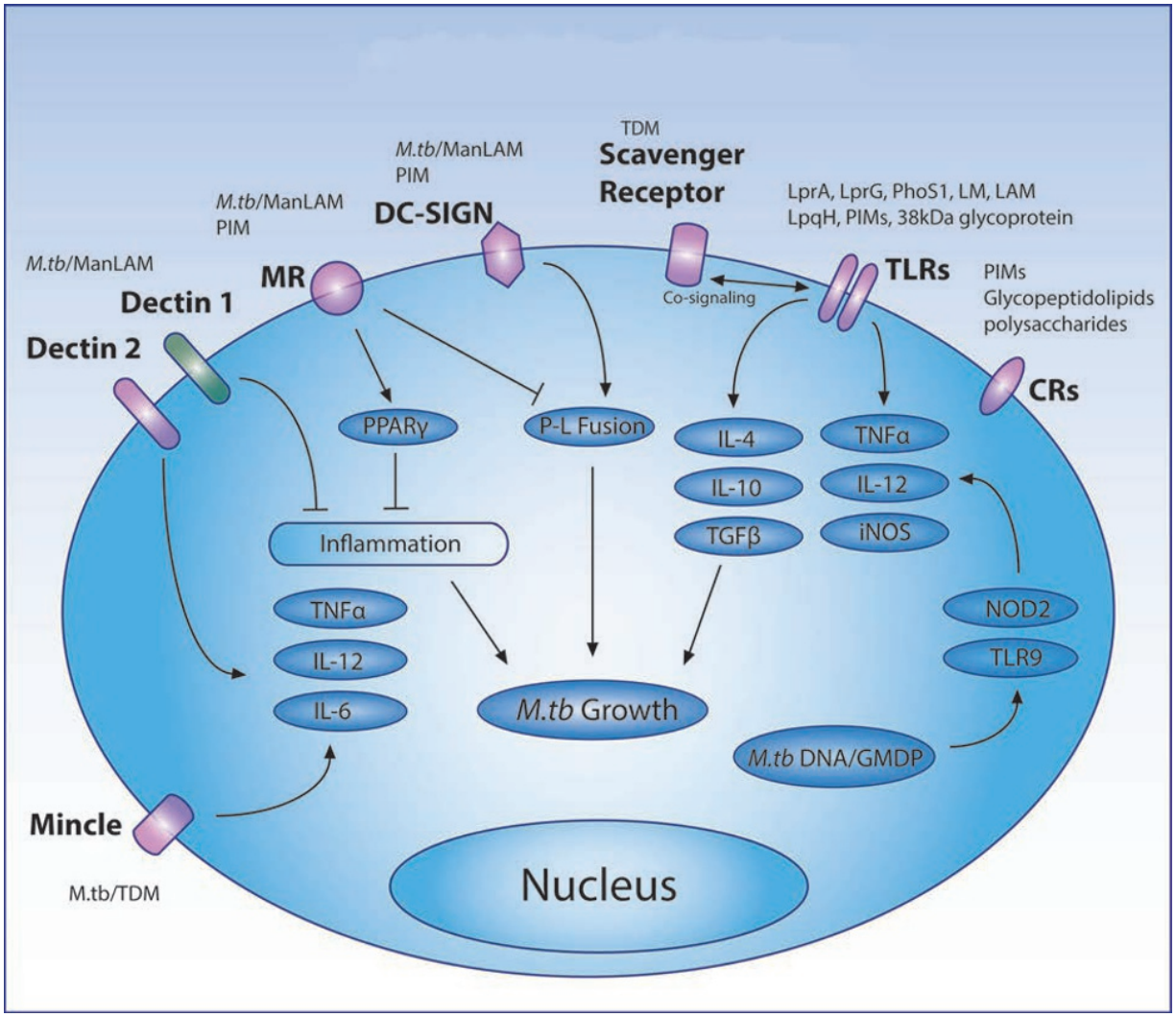

Figure 2 Macrophage receptors known to engage *M. tuberculosis* (*M.tb*) or its components and the downstream effects of receptor engagement on cytokine production, phagosome-lysosome fusion, and inflammation. Engagement of different receptors results in a macrophage response that can either promote or limit host immunity to *M. tuberculosis* infection.

containing lipids and is expressed by DC subsets and macrophages (115). Its stimulation by ManLAM induces pro- and anti-inflammatory responses and promotes T cell-mediated adaptive immunity in mice (116). Dectin-3 (also called MCL and Clec4d) recognizes TDM and is required for TDM-induced Mincle expression and production (117, 118).

Complement receptors CR1 (CD35), CR3 (CD11b/CD18), and CR4 (CD11c/CD18) are major phagocytic receptors expressed by monocytes, macrophages, neutrophils, and some lymphocytes, and their expression and activities change in a tissue- and differentiation-specific manner (Fig. 2). For example, CR4 expression increases during differentiation of monocytes into macrophages and is the prominent CR on AMs (119, 120). CRs mediate phagocytosis of opsonized and nonopsonized *M. tuberculosis* by human macrophages (67, 68, 121, 122) and recognize surface polysaccharides, PIMs, and glycopeptidolipids of nonopsonized *M. tuberculosis* (123, 124).

FcγRs play a role in the phagocytosis of *M. tuberculosis* following opsonization of bacteria with immune-specific antibody (67); this can lead to increased PL fusion (125).

TLRs

TLRs are a highly conserved family of transmembrane PRRs that are expressed by many cells, including AMs

and DCs (126–130) (Fig. 2). They are surface exposed (e.g., TLR2 and TLR4) and intracellular (e.g., TLR9) (127) and are classically thought of as proinflammatory through NFκB activation (131–133) but can also act through the negative regulators TRIF, IRF, and IRAK-M (134, 135). Recognition of *M. tuberculosis* occurs via TLRs 2, 4, and 9. Mycobacterial lipids (phosphor-*myo*-inositol-capped LAM, PIM_2, and PIM_6) and 19-kDa lipoprotein are TLR2 agonists (136, 137). TLR4 recognizes heat shock protein 65 (136), and TLR9 recognizes *M. tuberculosis* CpG motifs (138). Studies in TLR knockout mice with *M. tuberculosis* have yielded contradictory results. Single and double knockout mice exhibit a range of phenotypes in response to *M. tuberculosis* infection including enhanced mortality and defective IL-12p40 and IFN-γ responses (139–142). Conversely, triple knockout TLR2/4/9 mice exhibited no loss of protective T cell responses, and growth of *M. tuberculosis* was similar in wild-type and knockout mice (143). Myeloid differentiation factor 88 (MyD88), an adaptor used by most TLRs, was reported to be indispensable for control of mycobacterial growth (143, 144), although MyD88's role may be tied to its importance in IL-1β signaling (145).

SRs

There are at least eight classes of SRs, which cooperate with other receptors to mediate their function (146) (Fig. 2). AMs express at least four different SRs: the class A scavenger receptor-A isoforms I and II (SRA-I/II), macrophage receptor with collagenous structure (MARCO), and the class B receptor CD36. SRA-I/II bind to most polyanionic molecules (147, 148), MARCO removes unopsonized particles in the lung (149), and CD36 removes apoptotic cells and oxidized LDL (150). SRs mediate *M. tuberculosis* binding to macrophages (151). MARCO cooperates with TLR2 and CD14 to initiate cytokine release following recognition of *M. tuberculosis* TDM (152). CD36 contributes to foam cell generation during *M. tuberculosis* infection (153) and PPARγ production during BCG infection (154). PPARγ induces CD36 expression in human AMs (155). The specific role of different SRs during *M. tuberculosis* infection is unclear, likely due to a redundancy in scavenger receptor expression (156). Infection of SRA-I/II (157, 158) and MARCO (159) single knockout mice indicates that these SRs may play a role in limiting inflammation and resistance to pulmonary pathogens. CD36 knockout mice are more resistant to *M. tuberculosis* infection (160). Further work is needed to understand the role of SRs during *M. tuberculosis* infection.

Phagosome maturation

Typical phagosome maturation involves sequential fusion of the phagosome with early endosomes, late endosomes, and lysosomes, during which process the pH decreases to 4.5 to 5.0 through the actions of a vacuolar ATPase. The phagosome also acquires antimicrobial peptides and proteases, including cathepsins that are activated by the low pH in the phagosome. These factors all contribute to the bactericidal nature of the mature phagosome and mediate clearance of the ingested particle (161). However, some pathogens, including *M. tuberculosis*, modify the phagosome such that it becomes a niche for intracellular replication (Fig. 3). *M. tuberculosis* phagosomes do not fully acidify, reaching a pH of 6.2, and do not fuse with lysosomes (Rab5, but not Rab7, is acquired). Many *M. tuberculosis* components interfere with this maturation, including ESAT-6/CFP-10 (early secretory antigenic target 6/culture filtrate protein 10), SecA1/2, ManLAM, and TDM (102, 162–165). ManLAM inhibits PL fusion through the macrophage MR (87). Recent work showed that a mycobacterial lipoprotein, LprG, binds ManLAM and controls its distribution in the mycobacterial envelope. Mutants of *M. tuberculosis* lacking LprG have less ManLAM on their surface and are less able to inhibit PL fusion (166, 167).

It has been proposed that *M. tuberculosis* can escape the phagosome and reside in the cytosol. This is controversial, with debate as to whether *M. tuberculosis* replicates in the cytosol or is released in conjunction with macrophage cell death (168). Cytosolic localization has been proposed because experiments in the 1980s to 1990s provide electron microscopy images showing intracellular *M. tuberculosis* independent of phagosome membranes (169–171), and components of *M. tuberculosis* are detected in the cytosol following infection in a region of difference-1 (RD-1) and ESAT-6-dependent manner (172–174). An explanation for the latter observation, besides complete phagosome membrane dissolution, is that the RD-1-dependent ESX-1 secretion system perforates, but does not destroy, the phagosome membrane, resulting in a "leaky," or porous, phagosome (175, 176). This leaky phagosome would allow *M. tuberculosis* components access to the cytosol and explain how *M. tuberculosis* infection leads to activation of cytosolic immune sensors.

Autophagy

Macroautophagy is a major form of autophagy, hereafter referred to simply as autophagy, which involves the entrapment of cytosolic compounds into double-membrane vesicles that fuse with lysosomes to mediate

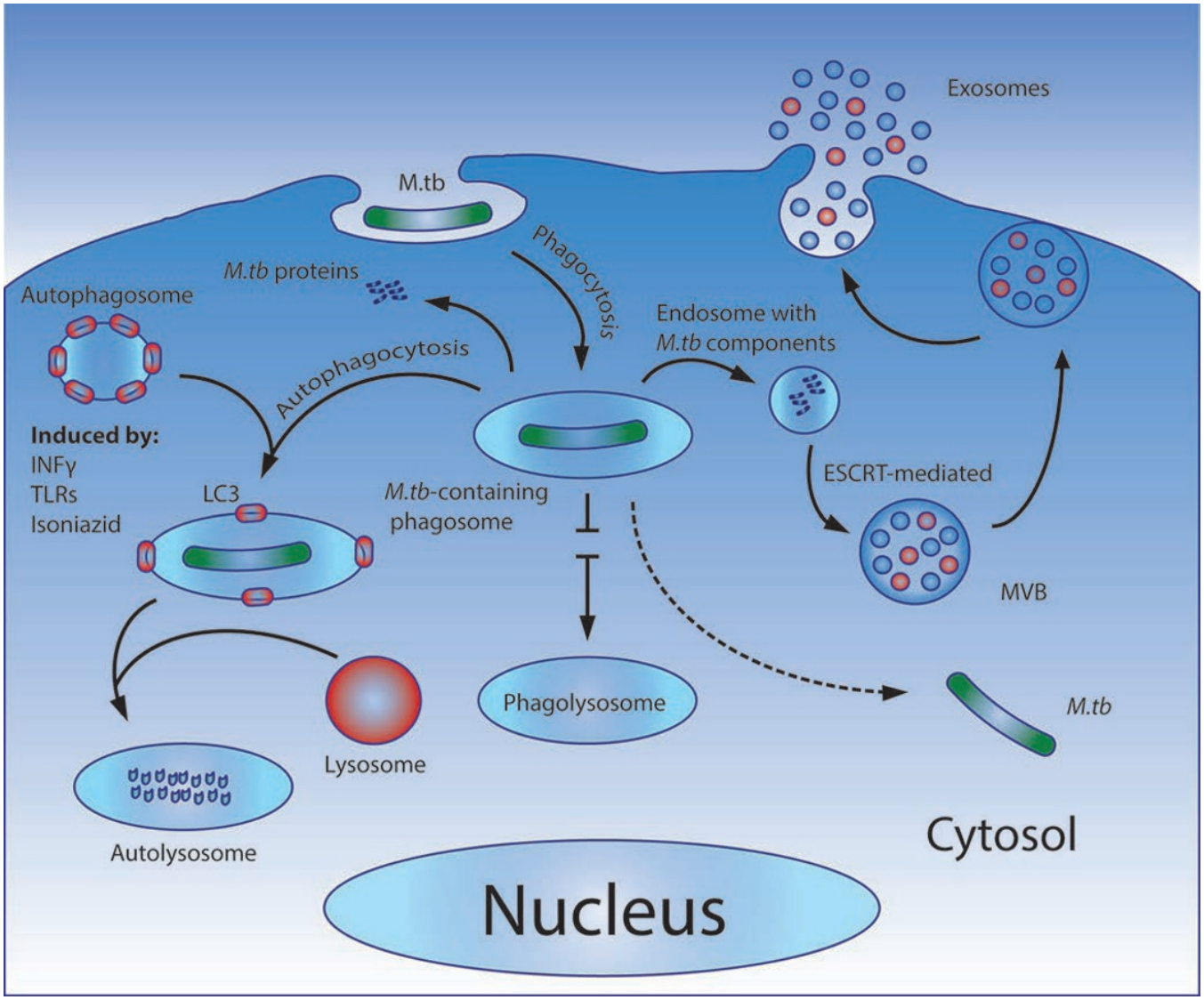

Figure 3 *M. tuberculosis* (*M.tb*) fate upon macrophage infection. Following phagocytosis, *M. tuberculosis* resides within a modified phagosome which may allow mycobacterial components to enter the cytosol in an ESX-1-dependent manner. The *M. tuberculosis* phagosome is also connected to the early endosomal network because membrane compartments can both fuse and bud from the phagosome, allowing exposure to important nutrients such as iron as well as removal of mycobacterial components. Endosomes containing mycobacterial components can fuse with multivesicular bodies (MVBs), leading to their incorporation into intraluminal vesicles, and upon MVB fusion with the plasma membrane, they can be released within exosomes (indicated as red circles in the figure). The *M. tuberculosis* phagosome has limited fusion with lysosomes, but with activation by IFN-γ or antibiotic treatment the *M. tuberculosis*-containing phagosome may undergo autophagosome formation and following lysosome fusion can limit *M. tuberculosis* growth, a process known as autophagy. There are also data suggesting that *M. tuberculosis* can escape into the cytosol, although this has been observed in only a limited number of studies.

degradation. This process is involved in cell maintenance and can also be used to limit infection (177–179). Starvation, rapamycin, TLRs, 2′-5′ cyclic GMP-AMP, IL-1, and IFN-γ can all induce autophagy (180). Recent publications have indicated that the host AMP-activated protein kinase-PPARγ, coactivator 1α pathway (AMPK-PPARGC1A; PGC-1), and membrane occupation and recognition nexus repeat containing 2 (MORN2) are involved in autophagy induction and regulate *M. tuberculosis* infection (181, 182) (Fig. 3). *M. tuberculosis* has various components that regulate autophagy, including ESAT-6 (176, 183) and the enhanced intracellular survival (*eis*) gene (184). If autophagy is induced, *M. tuberculosis* colocalizes with the autophagy marker LC3, PL fusion occurs, and *M. tuberculosis* growth is limited (185, 186). Autophagy is important *in vivo*; autophagy-deficient mice show increased *M. tuberculosis* growth, lung pathology, and reduced survival compared to wild-type mice (176, 187). Two frontline TB drugs, isoniazid and pyrazinamide, induce autophagy in *M. tuberculosis*-infected macrophages and are more inhibitory toward *M. tuberculosis* growth if the autophagy machinery is intact (188). Manipulation of autophagy is being pursued as a treatment option for TB (189–192).

Intracellular receptors

Intracellular receptors include the transmembrane TLRs and cytosolic nucleotide-binding oligomerization domain-containing protein (NOD)-like receptors (NLRs), RIG-I-like receptors, AIM2-like receptors (ALRs), and other cytosolic DNA sensors (193–197). Some of these receptors have been shown to recognize *M. tuberculosis* ligands and play a role during *M. tuberculosis* infection.

NOD1 recognizes D-glutamyl-meso-diaminopimelic acid, while NOD2 recognizes muramyl dipeptide (MDP) and a glycolated form of MDP (GMDP) produced by *M. tuberculosis* (198–200). NOD2 regulates *M. tuberculosis* growth in human and murine macrophages (201–203), perhaps via release of antimicrobial peptides and autophagy, since MDP increases LL-37, IRGM, and LC3 expression and *M. tuberculosis* killing in AMs (204). *M. tuberculosis* infection also activates NLRP3, but NLRP3 function during infection is unclear since NLRP3-deficient mice show a similar susceptibility to *M. tuberculosis* infection as wild-type mice. On the other hand, mice lacking the adaptor protein PYCARD/ASC, which (like NLRP3) is involved in caspase-1 activation, are more susceptible to *M. tuberculosis* infection (205). AIM2 and the ALR IFI204 recognize DNA and may play a role during *M. tuberculosis* infection (175, 206); AIM2-deficient mice have a higher bacterial burden and succumb to infection more quickly than wild-type mice (207).

PPARγ

The PPARs are a family of nuclear receptor-associated transcription factors. They include PPARα, PPARβ/δ, and PPARγ (208, 209). PPARγ is highly expressed by AMs, and its deletion leads to increased expression of IFN-γ, IL-12, macrophage inflammatory protein 1 alpha, and inducible nitric oxide synthase (210). PPARγ expression is induced in macrophages through the MR and TLR2 by *M. tuberculosis* and BCG, but not by the avirulent *M. smegmatis* (23, 211). PPARγ inhibition or knockdown leads to reduced *M. tuberculosis* intracellular replication and lipid body formation and enhanced TNF-α production (23, 153, 211). PPARγ is actively being pursued as a drug target, and efforts are ongoing to increase our understanding regarding its activities (209).

microRNAs (miRNAs)

miRNAs are endogenous, noncoding small RNAs that are typically transcribed from intergenic or intragenic regions of the genome in the pri-miRNA form. Following processing into miRNAs, they bind target mRNAs and typically mediate translational repression or mRNA degradation (212–214). Recent attention has focused on the specific regulation and function of miRNAs in the lung, particularly regarding cancer and inflammatory responses (215, 216). miRNAs serve several potential functions during *M. tuberculosis* infection: regulating TLR signaling, NFκB activation, cytokine release, autophagy, and apoptosis to alter *M. tuberculosis* infection and host survival (22, 217, 218). For example, miR-124 downregulates expression of MyD88, TRAF6, and TLR6 (219), and miR-let-7f targets a negative regulator of NFκB, A20, to increase cytokine and nitrite production and reduce *M. tuberculosis* infection (217). miR-132 and miR-26a negatively regulate the transcriptional coactivator p300 and IFN-γ signaling (220). Expression of miRNAs can be altered in *M. tuberculosis*-infected patients or cells (220–226), sometimes in a virulence-dependent manner; e.g., *M. tuberculosis*, but not *M. smegmatis*, infection induces expression of miR-125b (227). miRNA activity can also be cell-type specific, because miR-19a-3p may regulate expression of 5-lipoxygenase in primary human T cells, but not B cells (228). Targeting of miRNAs is a promising host-directed therapy (229).

Macrophage release of exosomes

miRNAs are also transported between cells via exosomes. Exosomes are membrane-bound vesicles of endo-

cytic origin that are released from most nucleated cells and function in intercellular communication and immune cell activation and serve as a source of disease biomarkers (230). In the context of an *M. tuberculosis* infection, exosomes released from infected macrophages can carry mycobacterial components including LAM, 19-KDa lipoprotein, and over 40 bacterial proteins, many of which are known immuno-dominant antigens (231, 232) (Fig. 3). The exosomes released from infected compared to uninfected macrophages also differ in their RNA content, and this has functional consequences on the exosome-recipient cell (233). Moreover, exosomes released from infected macrophages elicit both innate and acquired immune responses *in vitro* and *in vivo* and when used as a vaccine can protect mice against an aerosolized infection (231, 234, 235). However, whether exosomes released from infected macrophages function in regulating the immune response in humans during an *M. tuberculosis* infection remains an open question.

Macrophage cell death

Cell death can be an important step in controlling infection, and as such, many pathogens manipulate host cell death pathways to enhance their survival (236). The two cell death pathways that have been most studied are apoptosis, which is commonly thought of as anti-inflammatory and is characterized by retention of cell membrane integrity, and necrosis, which is typically proinflammatory and characterized by loss of membrane integrity (237, 238). Pyroptosis has characteristics of necrosis and apoptosis; pyroptotic cells lose membrane integrity similar to necrotic cells, but cell death is caspase-dependent, similar to apoptosis (239). Virulent *M. tuberculosis* inhibits apoptosis and instead induces necrosis to exacerbate infection. Apoptosis prevents *M. tuberculosis* dissemination and enhances antigen presentation to DCs and T cell priming. Necrosis and necroptosis mediate *M. tuberculosis* exit and dissemination from the infected cell, propagating infection; *M. tuberculosis* may downregulate pyroptosis, but this is not clear (240–242). *M. tuberculosis* components involved in regulating cell death include SodA, NuoG, ESX-1, and ESX-5 (243).

Interaction with DCs

DCs are a unique subset of immune cells which under steady-state conditions function as sentinels of the immune system. Immature DCs phagocytose *M. tuberculosis* at the site of infection, mature, migrate to secondary lymphoid organs, and prime T cells. DCs are equipped with a repertoire of PRRs for PAMPs and damage-associated molecular pattern molecules (DAMPs) (Fig. 4). Engagement of individual receptors can ultimately dictate downstream DC responses. TLRs 2, 4, and 9 and DC-SIGN recognize *M. tuberculosis* surface molecules, as discussed above. The interaction between DC-SIGN and ManLAM expressed on virulent mycobacteria is exploited by *M. tuberculosis* to its benefit, with *M. tuberculosis* using DC-SIGN as a portal into DCs; once engulfed, bacteria are targeted to late endosomes/lysosomes expressing lysosomal-associated membrane protein 1 (LAMP1) (100). DC-SIGN-mediated entry leads to IL-10 production and inhibition of DC maturation (100), which in turn causes inefficient T cell priming and a state of antigenic tolerance (244). Mycobacteria are able to persist in DCs (245, 246). Other mycobacterial products such as Hip1, a serine hydrolase, modulate DC responses and intracellular survival of the pathogen. Hip1 mutants of *M. tuberculosis* induce high levels of IL-12 and increased expression of MHC-II in a MyD88- and TLR2/9-dependent manner (247). *M. tuberculosis* is also able to retain coronin-1 on the vesicular membrane, which interferes with phagosome maturation and promotes survival of the pathogen (245). Lung DCs encompass three major subsets of cells: conventional DCs, plasmacytoid DCs, and monocyte-derived DCs, with each subset having specific and interrelating functions in the host (248). Data from mouse studies show that monocyte-derived DCs are rapid responders and are detected in the lung as early as 48 hours postinfection (249, 250). DCs undergo phenotypic changes following engulfment of mycobacteria which include upregulation of MHC-I/II, CD40, CD80, and CD86 (251); increased production of IL-12, TNF-α, IL-1, and IL-6 (252–254); and increased migration to lymph nodes for T cell antigen presentation (255).

In specialized microenvironments, migratory DCs are able to efficiently prime CD4 T cells (256) and activate CD8 T cells by the endosome-cytosol (257) or "detour" (258) antigen presentation pathway. DCs possess a pathway capable of transferring exogenous antigens from the endosome to the cytosol, leading to the presentation of antigens via the classical MHC-I pathway (173, 257). As part of the "detour pathway" uninfected DCs engulf apoptotic vesicles released from *M. tuberculosis*-infected macrophages and present antigens from these extracellular vesicles to CD8 T cells (258).

DCs (in addition to macrophages) are key initiators of the immune response and are thought of as Trojan horses providing a reservoir for *M. tuberculosis* to survive and escape immune surveillance (259, 260). Char-