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# MINIREVIEW

# Immunopathology of Tuberculosis: Roles of Macrophages and Monocytes

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## INTRODUCTION

Robert Koch identified *Mycobacterium tuberculosis* as the causative agent of tuberculosis (TB) in 1882 and was the first to realize that the efficacy of his early tuberculin therapies depended largely on the strength of the patient's immune response. Recent understanding of the roles played by leukocytes and the cytokines they secrete has revealed much about the delicate underlying balance between the strategies used by *M. tuberculosis* to survive within a host and the concomitant efforts of the host to kill it. In this article, we review the current state of understanding of the roles played by mononuclear phagocytes in response to inhaled *M. tuberculosis*.

Archeological evidence indicates that TB has afflicted humans for thousands of years (18). Following a 30-year period in which the incidence of TB in the United States declined by about 5% per year, this figure has begun to rise again. Between 1985 and 1992, TB cases in the United States increased by about 20% (8). This resurgence has been linked to the spread of human immunodeficiency virus (HIV) and to a combination of demographic and socioeconomic factors that have acted in concert to maintain a reservoir of infected persons. A recent report by the World Health Organization predicts that by the year 2005, TB will kill 4 million people annually. This is a significant increase from an estimated 3 million deaths worldwide caused by TB in 1992. While TB is a preventable and largely curable disease, our understanding of the cellular and molecular interactions between mycobacteria and host immune cells is far from complete, and the topic presents significant research challenges.

## THE MYCOBACTERIAL SURFACE

Few microorganisms can survive inside macrophages, due to the abundance of acidic phagocytic vacuoles and hydrolytic enzymes. *M. tuberculosis* bacilli are noteworthy for having evolved mechanisms that allow them to survive and multiply there. At least some of this success is thought to be linked to the unusual physicochemical properties of the myocobacterial surface (10). Figure 1 shows a schematic representation of the cell wall of *M. tuberculosis*. The bacterium is enclosed within a typical lipid bilayer cytoplasmic membrane, which lies beneath rigid peptidoglycan (PG). A number of proteins are found in association with PG and between the membrane and PG, and some of these may be immunogenic (3, 10). Continuing outward, PG is covalently linked via phosphodiester bonds to arabinogalactan (AG), a polymer of arabinose and galactose (1, 6). Mycolic acids, large ($C_{60}$ to $C_{90}$) branched-chain fatty acids, usually found as mixtures of homologs, are attached to the distal portion of the AG (80). The complex of PG, AG, and mycolates of the disaccharide trehalose (cord factor) are also associated with the cell wall skeleton. Another group of important cell wall components are the acylated trehalose-2′-sulfates. These may be important for virulence, since the most virulent strains of *M. tuberculosis* elaborate strongly acidic sulfolipids, which may be involved in inactivating the macrophage phagosome (6).

Another *M. tuberculosis* cell wall component that has been the focus of research interest is lipoarabinomannan (LAM), which, while anchored in the mycobacterial cell membrane, is thought to extend all the way to the surface. LAM is found as a heterogeneous mixture of arabinose- and mannose-containing phosphorylated high-molecular-weight lipopolysaccharides (e.g., 17 kDa) (10, 41, 85). The fatty acids, typically palmitate and tuberculostearate, occur in the form of diacylglycerol, linked to the branched arabinose- and mannose-containing polysaccharide via phosphatidyl-*myo*-inositol (not usually seen in prokaryotes) (6). The arabinose termini of LAM from *M. tuberculosis* and *Mycobacterium bovis* BCG (BCG) are capped with a few additional mannose residues (manLAM) in contrast to LAM from fast-growing nonpathogenic mycobacteria (AraLAM), in which the termini appear to be capped with additional inositol phosphates. This difference in capping significantly affects macrophage responses (16). These and other LAM influences on monocytes and macrophages are discussed in more detail below.

## PRIMARY PULMONARY TB

Once inhaled, fewer than 10% of *M. tuberculosis* organisms will reach the respiratory bronchioles and alveoli; most will settle in the upper respiratory epithelium, where they are likely to be expelled by the mucociliary escalator (49). Bacteria that arrive in the deep lung are phagocytosed by alveolar macrophages and either killed or else survive to initiate an infection (20, 65). Over the next 2 to 3 weeks, surviving organisms multiply and kill their host macrophages; this is followed by myobacterial release and subsequent infection of additional host cells. The early exudate contains chemotactic factors that attract circulating monocytes, lymphocytes, and neutrophils, none of which kills the bacteria very efficiently (78). Enhanced production of monocytes and their early release from bone marrow can be observed clinically (72). Granulomatous focal

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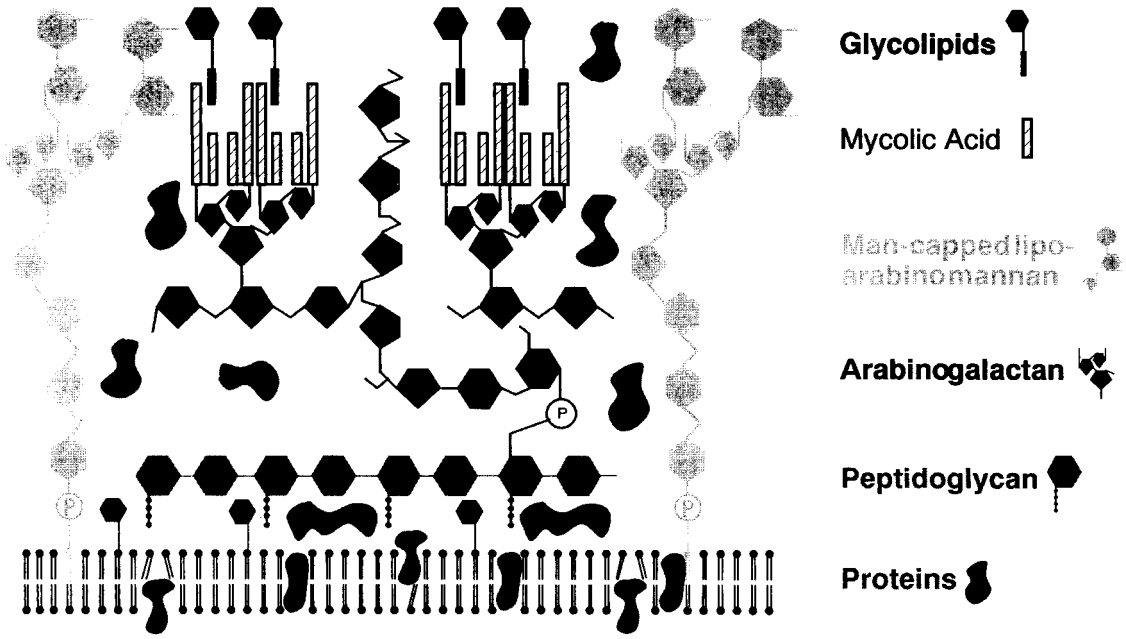



FIG. 1. Schematic representation of a cell wall of *M. tuberculosis*. Reprinted from reference 6 with permission of the publisher.

lesions, composed of macrophage-derived epithelioid giant cells and lymphocytes, begin to form. Generally, the process of granuloma formation serves as an effective means for containing pathogens, preventing their continued growth and dissemination. Its success depends on both the number of macrophages at the site of infection and on the number of organisms present. A host's initial resistance to *M. tuberculosis* infection is directly proportional to the strength of this granulomatous response. TB granulomas display a relatively high rate of monocyte and lymphocyte turnover, attesting to the toxicity of the *M. tuberculosis* bacilli for the host cells, which must be continuously replaced by fresh recruits (72 77). While granuloma formation is quite an effective defense, even contained *M. tuberculosis* organisms are not always completely eradicated.

Granuloma formation and destruction of mycobacteria by macrophages are not antigen-specific events, and heat-killed or living *M. tuberculosis* bacilli are equally efffective inducers of a granulomatous response (89). This observation contrasts with both delayed-type hypersensitivity (DTH) and cell-mediated immunity (CMI). In DTH, antigen-specific T-cell immune responses are evoked, and in CMI, live mycobacteria are required for the development of protective immunity (56). In the first few days following infection, a strong granulomatous response is vital. However, after about 3 weeks, antigen-specific defenses develop and contribute greatly to the resolution of infection. With the emergence of a DTH response, infected macrophages in the interior of each granuloma are killed as the periphery becomes fibrotic and caseated (78). After 4 to 5 weeks of progressive infection, microscopic granulomas enlarge, as individual foci expand and coalesce (49). This results in relatively large areas of necrotic debris, each surrounded by a layer of epithelioid histiocytes and multinucleated giant cells. These granulomas, or tubercles, are surrounded by a cellular zone of fibroblasts, lymphocytes, and blood-derived monocytes. Although *M. tuberculosis* bacilli are unable to multiply within this caseous tissue, due to its acidic pH, low availability of oxygen, and the presence of toxic fatty acids, some organisms may remain dormant there for decades. The strength of the host's CMI responses determines whether an infection is arrested here or progresses to the next stages.

With good CMI, the infection may be arrested permanently at this point. The granulomas subsequently heal, leaving small fibrous and calcified lesions (49, 78). However, if CMI responses are insufficient, macrophages containing ingested but viable *M. tuberculosis* organisms may escape from the granuloma via the intrapulmonary lymphatic channels. This results in the rapid spread of the infection to the regional hilar lymph nodes. Where CMI is inadequate, the host's DTH responses battle the ever-multiplying *M. tuberculosis* bacilli, but concomitantly, lung tissue is destroyed, leading to both pulmonary damage and the spread of organisms via the lymphatics and the blood. As disease progresses further, the semisolid caseous center of the granuloma begins to soften and liquefy, providing a rich and oxygenated environment for extracellular mycobacterial replication (19). Enlarged lymph nodes can rupture into adjacent airways, releasing liquified necrotic material and causing tuberculous bronchopneumonia (33). At this point, all patients require effective antibiotic therapy in order to survive (20).

## POST-PRIMARY PULMONARY TUBERCULOSIS

There are two routes to a repeat episode of TB (post-primary pulmonary TB): either by inhalation of additonal *M. tuberculosis* organisms or by reactivation of a dormant primary lesion. The existence of post-primary TB means that an infection can proceed in spite of existing immunity. In reinfection TB, a hypersensitivity reaction is the characteristic response, accompanied by tissue necrosis and caseation (90). In an attempt to seal off the necrotic site, lymphocytes and other cells converge upon the site and direct formation of a wall of fibrous tissue (78). Some lymphatic spread may occur, but in this case, the hilar lymph nodes are not implicated. Most often, caseated granulomas heal over time, shrinking as they become fibrotic and then calcified. However, if healing is impaired, the

growing lesions may erode adjacent bronchi, resulting in the formation of cavities. *M. tuberculosis* organisms multiply freely in these cavities, leading to huge numbers of bacilli (estimated at greater than $10^8$) (49). An open cavitated lesion can leak infectious material directly into the bronchus, resulting in the continuous discharge of bacilli into the sputum (78). Leaked *M. tuberculosis* bacilli can also be inhaled into other portions of the host's lungs, resulting in tuberculous bronchopneumonia. If the growing granulomatous lesion erodes the wall of a vein, organisms can spread in the circulating blood, resulting in miliary disease (40).

*M. tuberculosis* bacilli can persist for decades in a dormant state inside a granuloma, particularly in the apical regions of the lung. Reactivation of these latent organisms can also lead to post-primary disease, even in persons who successfully fought their initial battle against TB. The majority of post-primary TB cases in the United States are thought to be episodes of reactivation. The mechanisms which govern dormancy and reactivation, within either the organism or the host, are not yet understood. The host typically mounts an inflammatory response to reactivation TB, with the lesion appearing circumscribed and necrotic (90). Reactivation TB that progresses to the cavitary stage favors the propagation of virulent and drug-resistant strains, as the increased oxygen concentration allows multiplication of the organisms, and large numbers are thought to be necessary for the evolution of drug resistance mutants (49).

## BINDING AND UPTAKE OF *M. TUBERCULOSIS*

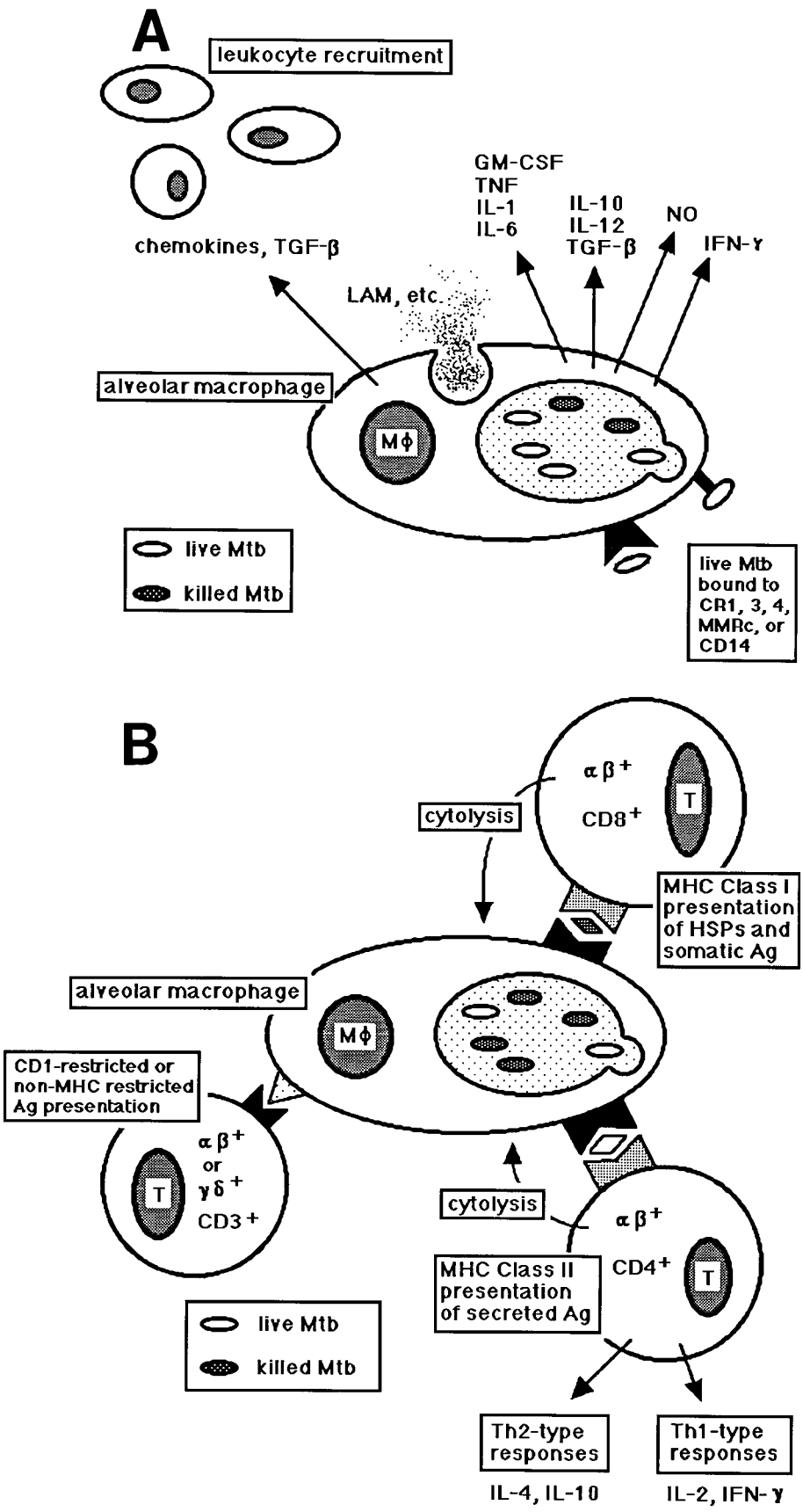
*M. tuberculosis* bacilli are thought to enter the macrophage via specific binding to several distinct cell surface molecules, and the precise route of pathogen entry is likely to determine the ultimate fate of bacilli within the macrophage. *M. tuberculosis* can bind directly via complement receptors and the macrophage mannose receptor (MMRc). The MMRc participates in nonopsonin-mediated phagocytosis by recognition of terminal mannose residues on targeted particles (reviewed in reference 83). Macrophage phagocytosis of virulent strains of *M. tuberculosis* (e.g., Erdman and H37Rv) in the absence of serum can be substantially inhibited by soluble mannan, mannose-albumin, and anti-MMRc antibodies (68). Binding of the attenuated *M. tuberculosis* strain H37Ra to macrophages is not blocked by these agents. Expression of MMRc on the cell surface is regulated by a variety of mediators that play a role in the pathogenesis of TB. Gamma interferon (IFN-γ) has been shown to down-regulate MMRc expression while concomitantly increasing the capacity of the cells to kill microorganisms, suggesting enhanced coupling of the MMRc to microbicidal functions (47, 74). In contrast, interleukin-4 (IL-4) is a potent enhancer of MMRc expression (81). The binding of virulent *M. tuberculosis* to the MMRc may be mediated by ManLAM, as suggested by the finding that anti-ManLAM monoclonal antibodies reduced *M. tuberculosis* binding to macrophages by as much as 49% (71). A mannose-dependent pathway for particle uptake by macrophages, distinct from the MMRc, is reportedly mediated by lung surfactant protein A (46). The possibility that surfactant protein A may serve as an opsonin for inhaled organisms, and may provide an alternative means for mycobacteria to enter the macrophage, remains to be tested. Mannose-dependent binding is likely to account for only a portion of total phagocytosed *M. tuberculosis*. A recent study has implicated CD14, a signaling receptor for gram-negative bacterial lipopolysaccharide (LPS), in the uptake of nonopsonized *M. tuberculosis* (61). Treatment of human microglial cells with anti-CD14 antibodies or soluble CD14 was found to significantly block the infection of these cells by *M. tuberculosis*. It is currently unclear if this uptake is mediated by LAM, a known ligand for CD14 (62). An important role for heat-labile serum components and the complement receptors CR1 (CD35), CR3 (CD11b/CD18), and CR4 (CD11c/CD18) in the binding of *M. tuberculosis* to macrophages has also been well established. Both adherence and ingestion of *M. tuberculosis* can be markedly inhibited (up to 84%) by antibodies against CR1, CR3, and CR4 in the presence or absence of fresh serum (68–70). Uptake of *M. tuberculosis* is also reduced in serum depleted of the complement component 3 (C3). Anti-C3 monoclonal antibodies were reported to inhibit monocyte adherence of preopsonized *M. tuberculosis* by 71% (69), demonstrating that C3 serves to facilitate *M. tuberculosis* binding to complement receptors. In contrast to the clear-cut role for complement receptors in *M. tuberculosis* adherence and uptake in the presence of nonimmune serum, the contribution of Fc receptor-mediated uptake remains uncertain. Because engagement of Fc receptors initiates the production of reactive oxygen intermediates (ROI), it is unlikely that successful macrophage pathogens would utilize Fc receptors as a means for infection.

## INTRACELLULAR FATE OF *M. TUBERCULOSIS*

Following attachment and subsequent phagocytosis of *M. tuberculosis*, sustained intracellular bacterial growth depends on the ability to avoid destruction by lysosomal enzymes, ROI, and reactive nitrogen intermediates (RNI). A capacity to block the fusion of mycobacterium-containing phagosomes with lysosomes could be critical for this survival; a key question is whether *M. tuberculosis* bacilli possess one. Recent electron microscopic studies have examined the dynamics of phagosome-lysosome fusion and its effect on intracellular bacterial replication in infected human macrophages (48). Vesicles containing viable *M. tuberculosis* appeared to bud from the phagosomes with no subsequent fusion with lysosomes. Furthermore, only viable and virulent *M. tuberculosis* displayed this capacity. Importantly, *M. tuberculosis* appears to have the ability to disrupt the normal functioning of phagosomes, preventing them from developing into acidic hydrolase-rich compartments. Several laboratories have reported a failure of mycobacterium-containing vesicles to fuse with endosomal vesicles containing other ingested material, such as electron-dense colloids (34, 38, 63). This restricted capacity of mycobacterial phagosomes to fuse with other vesicles suggested that their biochemical composition is altered, either preventing delivery of the mycobacteria into the lysosomal compartment or blocking association of phagosomes with host molecules that are harmful to the bacilli. The latter possibility was confirmed by studies that revealed that vacuolar membranes surrounding the bacilli lacked a proton-ATPase, which may be responsible for phagosomal acidification (82, 88). Moreover, containment of viable *M. tuberculosis* within these specialized vesicles may reduce the capacity of mycobacterial antigens to be processed, associated with major histocompatibility complex (MHC) class II proteins, and/or transported to the cell surface (60). While one study also reported the presence of free *M. tuberculosis* within the macrophage cytosol (48), other investigators have been unable to replicate this finding (82, 88).

## MICROBICIDAL ACTION OF MACROPHAGES

Historically, researchers have assumed that activated macrophages can kill *M. tuberculosis*. However, this assumption has been difficult to prove unequivocally in vitro, especially with


A
leukocyte recruitment
GM-CSF
TNF
IL-1
IL-6
IL-10
IL-12
TGF-β
NO
IFN-γ
chemokines, TGF-β
LAM, etc.
alveolar macrophage
Mϕ
live Mtb
killed Mtb
live Mtb bound to CR1, 3, 4, MMRc, or CD14
B
cytolysis
αβ+
CD8+
T
MHC Class I presentation of HSPs and somatic Ag
alveolar macrophage
Mϕ
CD1-restricted or non-MHC restricted Ag presentation
αβ+ or γδ+
CD3+
T
cytolysis
αβ+
CD4+
T
MHC Class II presentation of secreted Ag
live Mtb
killed Mtb
Th2-type responses
IL-4, IL-10
Th1-type responses
IL-2, IFN-γ

human monocytes and macrophages. Crowle's group reported that human monocytes cultured for 3 days were measurably better at suppressing the growth of virulent *M. tuberculosis* than were either fresh monocytes or those cultured for 7 days (28). Another researcher reported that human monocytes could be activated to high microbicidal activity following treatment with cytokines such as IFN-γ and tumor necrosis factor (TNF) (21). However, a more recent report suggested that this apparent killing of *M. tuberculosis* could be an artifact of the experiment and that this cytokine treatment regimen actually renders macrophages more sensitive to the toxic effects of the mycobacteria (86).

Should it occur, killing of ingested *M. tuberculosis* would most likely take place within macrophage phagolysosomes. Toxic constituents found within this acidic vesicle include lysosomal hydrolases, ROI such as $H_2O_2$ and $O_2^-$, and RNI such as NO and $NO_2^-$. The resistance of several strains of *M. tuberculosis* to RNI in vitro, generated at an acidic pH, was found to correlate significantly with the virulence of the strain tested (54). RNI production by murine macrophages is an important effector mechanism against a variety of pathogens (reviewed in reference 50). In macrophages, NO and other RNI are derived from L-arginine via an enzymatic pathway controlled by an inducible nitric oxide synthase (iNOS) (reviewed in reference 51). Cytokines are powerful modulators of murine macrophage RNI synthesis. While TNF and IFN-γ are potent activators of iNOS, IL-4 and IL-10 suppress it (30, 36, 58). Transforming growth factor β1 (TGF-β1) has also been reported to attenuate NO production and RNI-mediated antimicrobial functions (26, 52, 58). Growth inhibition of mycobacteria by cytokine-stimulated murine macrophages strongly correlates with the generation of RNI (14, 15, 23, 29). IFN-γ-deficient mice infected with *M. tuberculosis* are unable to restrict the growth of the organisms. These mice can develop granulomas, but fail to produce RNI (32).

The role of RNI in infected humans remains a matter of considerable debate (24). Production of iNOS mRNA (64) and protein (45) by primary human macrophages has been reported, although efforts to demonstrate an L-arginine-dependent pathway for RNI production in human macrophages have generally produced inconsistent results. These disparate observations may simply reflect intrinsic differences between human and murine cells. One possible difference may be the requirement for the cofactor tetrahydrobiopterin, which may not be present in sufficient quantities in resting human macrophages (73, 87). Alternatively, human macrophages may require additional induction signals for RNI production. One study showed that a combination of LPS and the cytokines IL-1, TNF, and IFN-γ was required in order for human hepatocytes to produce RNI via an L-arginine-dependent pathway (53). Furthermore, this iNOS activity was dependent on the coinduction of tetrahydrobiopterin synthesis (27). Another possibility is that human macrophages may produce higher amounts of cytokines that suppress RNI production (e.g., IL-4, IL-10, and TGF-β1) compared with murine cells. Lastly, the contribution of ROI to the defense against TB remains unclear. ROI alone may be insufficient to destroy *M. tuberculosis*, but ROI combined with RNI can significantly enhance mycobacterial killing (79, 95). Several mycobacterial products, including sulfatides and LAM, can scavenge ROI or inhibit the respiratory burst that generates them (13, 59).

The capacities of resident alveolar macrophages and recruited monocytes to destroy mycobacteria differ significantly and progressively throughout the course of an infection. Initial infection by *M. tuberculosis* rapidly leads to the activation of alveolar macrophages, the induction of cytokines which serve to limit the growth of the ingested organisms, and the recruitment of additional leukocytes from the peripheral circulation. Enhancement of macrophage microbicidal function can be exerted by T cells, especially $CD4^+$ $\alpha\beta^+$ T cells that secrete IFN-γ and IL-2 (56). IFN-γ alone cannot activate macrophages sufficiently to inhibit *M. tuberculosis* replication, although combined exposure to IFN-γ and TNF (and possibly additional factors) was reported to be sufficient for effective killing (21). While activated alveolar macrophages may kill *M. tuberculosis* effectively, immature monocytes recruited from the periphery are thought to be less effective, serving as the organism's preferred hosts. The local production of IFN-γ and TNF by leukocytes is critical for the differentiation and activation of these recruited monocytes. Treatment of mice infected with BCG or *M. tuberculosis* with a neutralizing antibody directed against TNF was originally shown to block granuloma formation and to enhance bacterial growth (22, 44). More recent work with transgenic mice lacking the 55-kDa TNF receptor demonstrated that these animals cannot clear an *M. tuberculosis* infection. These mice and others treated in vivo with anti-TNF neutralizing antibody were reported to form granulomas, but in a delayed manner. In addition, the granulomas observed in mice lacking normal TNF function were qualitatively distinct, lacking epithelioid cells and containing larger numbers of *M. tuberculosis* (31). Other macrophage-derived cytokines that may activate human cells to kill mycobacteria include granulocyte-macrophage colony-stimulating factor and IL-12 (7, 25, 35, 91). In contrast, IL-1 and IL-6 have been reported to increase intracellular mycobacterial growth (25, 75).

## EFFECT OF LAM ON MACROPHAGES

Recent studies suggest that *M. tuberculosis* bacilli which have evaded destruction by the macrophage not only multiply but may release mycobacterial products that can affect local im-

FIG. 2. (A) Macrophage responses to *M. tuberculosis*. Present evidence suggests that alveolar macrophages serve as the primary hosts for *M. tuberculosis*. Specific binding and receptor-mediated uptake of bacilli can be mediated by a variety of cell surface proteins including the complement receptors (CR) 1, 3, and 4, the macrophage mannose receptor (MMRc), and CD14. Internalized *M. tuberculosis* can grow progressively in specialized endosomal vesicles which do not become acidified. While some mycobacterial antigens are sequestered within these vesicles, LAM and some proteins secreted by viable bacilli are released by the infected macrophage. Infection induces the expression of chemokines which serve to recruit monocytes, neutrophils, and T cells to the site of infection. Other cytokines, such as TNF, appear to be critical for containing the infection. Other cytokines (e.g., IL-10, IL-12, and TGF-β) can influence the development of subsequent Th1- and Th2-type T-cell responses. Nitric oxide (NO) produced by murine macrophages is an important microbicidal factor, although its role in human TB remains unclear. Unpublished data from the authors' laboratories have shown that human alveolar macrophages can secrete IFN-γ in response to *M. tuberculosis* infection in vitro. IFN-γ may function as an autocrine priming signal to augment macrophage microbicidal activities. (B) Interactions between lymphocytes and *M. tuberculosis*-infected macrophages. As infection progresses, mycobacterial antigens are processed and presented by the macrophages. Peptide antigens derived from proteins secreted by viable *M. tuberculosis* are recognized by MHC class II-specific $CD4^+$ αβ T cells, whereas peptide antigens derived from heat shock proteins (HSPs) and bacterial somatic proteins are recognized by MHC class I-specific $CD8^+$ αβ T cells. Both types of T cells can become cytotoxic for macrophages presenting mycobacterial antigens. In the case of $CD4^+$ cells, Th1-type responses are diminished during the development of TB, whereas Th2-type responses are either unaffected or are enhanced. Double-negative T cells ($CD4^-$ $CD8^-$) have recently been shown to recognize nonpeptide mycobacterial antigens, such as LAM and mycolic acid, in an MHC-independent but CD1-restricted manner.

mune responses. Vesicles containing LAM (and possibly other mycobacterial products) were released by phagosomes containing ingested mycobacteria, suggesting the active transport of mycobacterial products out of infected cells (82, 88). LAM released by infected macrophages may act in a paracrine manner to modulate the function of surrounding leukocytes. Several studies have examined the biological activities of LAMs from different mycobacteria and defined chemical derivatives of LAM. AraLAM is 100-fold more potent at inducing TNF production by macrophages than is ManLAM (16). Similar results have been observed for IL-1, IL-10, NO, and chemokine induction (66, 67, 94). Underpinning these results is the recent finding that AraLAM rapidly activates the critical transcription factor NF-κB in murine macrophages to a much greater extent than does ManLAM. Both AraLAM and ManLAM induce sustained accumulation of KBF1, which may inhibit NF-κB-dependent gene transcription (11). While reports differ with respect to the observed biological importance of polysaccharide domains at the nonreducing termini of LAM, there was general agreement that acyl groups associated with the phosphatidylinositol end of the molecule are essential for the biological activities of LAM on monocytic cells (2, 16, 93). In contrast, we have recently observed that both AraLAM and ManLAM can directly induce human T-cell chemotaxis in vitro (4a). As noted above, LAM may bind to the MMRc, but a recent study reported that LAM can also directly insert into leukocyte membranes and selectively affect the mobility of cell surface proteins, a property dependent on its acyl side chain moieties (42).

## INTERACTIONS BETWEEN T CELLS AND MACROPHAGES

Collaboration between macrophages and T cells is essential for eradication of *M. tuberculosis* via antigen-specific DTH responses. Initially, activated macrophages present mycobacterial antigens to T cells in association with MHC class I or class II proteins or with nonpolymorphic MHC-like proteins (e.g., CD1). Macrophage-derived cytokines play critical roles as costimulatory molecules for antigen-specific T cells. Subsequently, cytokines produced by activated T cells further modulate macrophage function, although the net result may be either to inhibit or to augment intracellular *M. tuberculosis* growth. Th1-type cytokines (e.g., IL-2 and IFN-γ) may enhance antigen presentation and stimulate antimicrobial responses within the infected macrophage itself. The presence of both antigen-specific T cells and activated macrophages within the granuloma provides long-term surveillance and containment of infected macrophages, although immune responses may shift over time to favor the suppression of DTH reactions. In murine models of TB, the initial Th1-type response needed for effective DTH development is followed by a Th2-type response which probably serves to limit inflammation and minimize tissue injury at the site of infection (57). The macrophage cytokine IL-12 may be important in favoring the development of the DTH response by enhancing production of IFN-γ, facilitating the development of Th1 cells, and augmenting the cytotoxicity of antigen-specific T cells and natural killer cells (reviewed in reference 12). Elevated IL-12 levels have been observed in pleural fluids obtained from tuberculous pleuritis patients (91). In contrast, Th2-type responses may contribute to the immunosuppression often observed in advanced disease. This is particularly apparent in the context of HIV: TB patients coinfected with HIV have diminished Th1-type responses compared with HIV-seronegative TB patients (92). Macrophages are likely to contribute further to these responses through the production of the immunosuppressive cytokines IL-10 and TGF-β1 (2, 5, 84). Thus, the cytokine network controls a complex set of responses that may be growth modulatory to *M. tuberculosis*, proinflammatory, or immunosuppressive. Some of these interactions are outlined in Fig. 2.

## PRESENTATION OF *M. TUBERCULOSIS* ANTIGENS BY MACROPHAGES

Macrophages serve as important antigen-presenting cells in the host response to *M. tuberculosis*. Mycobacterial antigens expressed in association with MHC class II or class I molecules can be recognized by $CD4^+$ and $CD8^+$ αβ $TCR^+$ T cells, respectively. MHC-independent recognition of *M. tuberculosis* antigens may be characteristic of the response of γδ $TCR^+$ T cells. The physiological role of γδ T cells in TB immunity has been the focus of much recent investigation. Human γδ T-cell lines which recognize mycobacterial antigens have been derived from the peripheral blood of a healthy tuberculin responder (37). These γδ T cells accumulate in substantial numbers in mycobacterial lesions and exhibit strong reactivity toward mycobacterial antigens (43). Viable *M. tuberculosis* bacilli induce the replication of human peripheral blood T cells in a manner that favors the expansion of γδ T cells (9, 39). In contrast, purified protein derivative from *M. tuberculosis* predominantly induces a $CD4^+$ αβ T-cell response with little or no increase in γδ T cells. A small (500- to 600-dalton) nonpeptide mycobacterial antigen has been identified from crude *M. tuberculosis* extracts based on its ability to induce the proliferation of $Vγ9^+$ $Vδ2^+$ T cells from healthy donors (17). This finding supports the hypothesis that some γδ T cells can recognize nonpeptide ligands. This remarkable possibility has been expanded to include αβ T cells. CD1b-restricted presentation of both mycolic acids (4) and LAM (76) to $CD4^-$ $CD8^-$ αβ T-cell clones has recently been demonstrated. Macrophage processing of these antigens is required for T-cell activation, as demonstrated by the failure of paraformaldehyde-fixed macrophages to induce proliferation of T-cell clones in the presence of exogenous antigen. Lastly, evidence has also been reported for a mycobacterial superantigen that induces MHC class II-dependent (and processing-independent) expansion of $Vβ8^+$ human T cells obtained from purified protein derivative-negative healthy donors (55).

## FUTURE DIRECTIONS IN TB RESEARCH

The renewed interest in TB research has led to a significant burst of activity in exploring the cellular and molecular level details of infection and immunity. Still, many important questions remain. Areas ripe for further exploration include additional mechanisms for binding and uptake of *M. tuberculosis* into alveolar macrophages, the importance of functional heterogeneity among macrophages, the manner by which *M. tuberculosis* disables host ROI and/or RNI microbicidal efforts, mechanisms by which normal phagosome development and fusion with lysosomes are blocked, alterations in antigen processing and presentation, potential contributions of epithelial and endothelial cells to the resolution or progression of *M. tuberculosis* infection, and disruptions of lymphocyte functions and cytokine production patterns. A great deal of challenging work remains if humans are to outwit this ancient and clever adversary.

An expanded discussion of the immunopathology of tuberculosis will be presented elsewhere (85a).

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