The Immune Response in Tuberculosis

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Abstract
There are 9 million cases of active tuberculosis reported annually; however, an estimated one-third of the world’s population is infected with Mycobacterium tuberculosis and remains asymptomatic. Of these latent individuals, only 5–10% will develop active tuberculosis disease in their lifetime. CD4+ T cells, as well as the cytokines IL-12, IFN-γ, and TNF, are critical in the control of Mycobacterium tuberculosis infection, but the host factors that determine why some individuals are protected from infection while others go on to develop disease are unclear. Genetic factors of the host and of the pathogen itself may be associated with an increased risk of patients developing active tuberculosis. This review aims to summarize what we know about the immune response in tuberculosis, in human disease, and in a range of experimental models, all of which are essential to advancing our mechanistic knowledge base of the host-pathogen interactions that influence disease outcome.
THE PROBLEMS OF TUBERCULOSIS AS A HUMAN DISEASE

Tuberculosis (TB), although largely a curable disease, still remains a major cause of morbidity and mortality worldwide. There were 9 million new cases and 1.4 million deaths in 2010 (1), despite various strategies implemented to tackle this global threat to human health (The Global Plan to Stop TB; http://www.stoptb.org) (reviewed in 2). The disease is caused by infection via the lung with the acid-fast bacillus Mycobacterium tuberculosis, first identified as a pathogen by Robert Koch in 1882 (3). TB is predominantly a disease of the lung, with pulmonary TB accounting for 70% of cases, although M. tuberculosis can disseminate to other organs, including lymph nodes, bone, and meninges, and cause extrapulmonary disease (4, 5). Most interestingly from an immunologist’s point of view, although 9 million new cases of active TB are still reported annually, an estimated one-third of the world is infected with M. tuberculosis but remains asymptomatic—defined as having latent TB (6). Of those with latent TB, only 5–10% will develop active TB disease in their lifetimes (7, 8) (Figure 1).

Control of the global TB epidemic has been impaired by the lack of an effective vaccine (9, 10), by the emergence of drug-resistant forms of M. tuberculosis, and by the lack of sensitive and rapid diagnostics (2). In addition, the immune response to M. tuberculosis is complex and incompletely characterized, which hampers attempts to develop new tests, vaccines, and treatments. Although it is evident from human disease and from experimental mouse models that CD4+ T cells (11–13) in addition to IL-12, IFN-γ (11, 12, 14, 15), and TNF (16, 17) are all fundamental in the control of M. tuberculosis infection, there remains an incomplete understanding of the host factors that determine why some individuals are protected from M. tuberculosis infection while others go on to develop disease (18). A recent report showed that, during Mycobacterium bovis bacillus Calmette-Guérin (BCG) vaccination of newborns, the frequency and cytokine profile of mycobacteria-specific T cells did not correlate with protection from or susceptibility to the subsequent development of TB (19). Critical components of immunity against M. tuberculosis, such as IFN-γ production by CD4+ T cells, may not translate into immune correlates of protection against disease (19). Thus, there is a lack of correlates of protection, which are needed to help predict the
Development of TB disease results from interactions among the environment, the host, and the pathogen, and known risk factors include HIV coinfection, immunodeficiency, diabetes mellitus, overcrowding, malnutrition, and general poverty (2). Capitalizing on the known host and bacterial factors that influence M. tuberculosis exposure outcomes, investigators have produced recent data suggesting that particular combinations of host (14, 15, 20, 21) and M. tuberculosis genotypes (22–26) are associated with increased risk of developing active TB and with disease severity. This review aims to summarize the known immune responses in TB, drawing on information obtained from human disease and experimental models. Because the disease is complex and heterogeneous, reflecting the various factors that can influence whether an individual infected with M. tuberculosis remains healthy or develops active TB, we first outline the spectrum of human TB and the difficulties in management of the disease.

THE SPECTRUM OF ACTIVE AND LATENT TUBERCULOSIS

Active Tuberculosis

Active TB encompasses a heterogeneous range of presentations and forms of disease. Classically, TB pathogenesis can be divided into two stages, each of which can present as active disease. Following initial infection with M. tuberculosis, some individuals progress rapidly to active disease, usually referred to as primary or primary-progressive TB, which is more common in children but also affects adults (27). In others, who contain the initial infection and are thereafter presumed to be latently infected, active disease can present after an interval of many years following exposure, with latent individuals having a 5–10% lifetime risk of developing active TB, termed reactivation or postprimary TB (Figure 1). Primary and postprimary TB may have distinct clinical presentations, with different temporal pathogeneses, and are proposed to represent differing host genetic susceptibilities (14, 15). Because of the significant variation among active TB patients, this complex disease is often underestimated and oversimplified. The combination of symptoms and examination findings may range from systemic responses such as fever, weight loss, and night sweats, to local consequences of the infection such as cough and hemoptysis in pulmonary disease (28), to radiological abnormalities such as thoracic lymphadenopathy and lung cavities or densities (29). These symptoms likely reflect the host response to the pathogen. Linking these clinical features to our knowledge of the molecular pathways of innate and adaptive immune effector functions may help us design strategies for elucidating the host factors underlying this complex disease.

Despite such overt clinical presentation, confirming the diagnosis of active TB disease can be difficult, but confirmation is essential. The classical clinical presentation of TB is nonspecific and overlaps with diseases such as pneumonia, lung cancer, and sarcoidosis, leading to delays before a practitioner even considers a diagnosis of TB (30). In pulmonary TB, demonstrating the presence of mycobacteria in the sputum by microscopy examination (so-called smear test positivity) has a variable sensitivity of between 32% and 97%, depending on the technique used, and does not distinguish between M. tuberculosis and nontubercular mycobacteria (31). Diagnosis thus requires isolation and confirmation of M. tuberculosis by culture, which can take up to 6 weeks (32), although the WHO recently endorsed the Xpert MTB/RIF automated molecular PCR-based test for M. tuberculosis and rifampicin resistance that gives a result within hours (33). In pulmonary TB patients in whom culture or microscopy of sputum is not available (34) (between 30% and 50%) or in those who have extrapulmonary disease, additional sampling may be required by an invasive procedure, such as bronchoscopy or biopsy (35), which is not always possible in countries with a high TB burden. The suboptimal performance of currently available tests relates directly to delays...
in diagnosis and thus to control of the disease (36).

An additional burden is that treatment of active disease requires the use of multiple drugs to prevent the selection of drug-resistant mutants from within the bacterial population. The treatment is lengthy—a minimum of 6 months—divided into an initial intensive phase to kill actively replicating bacilli, followed by a continuation phase to ensure that persisting bacilli are also targeted (37, 38). Furthermore, the drugs have appreciable toxicity, most commonly hepatotoxicity (5%) (39). After diagnosis, no early biomarkers correlating with treatment success exist, resulting in a significant delay in assessing treatment response. Conversion to negative culture from sputum after 2 months of treatment is the only accepted biomarker (40). However, a systematic review and meta-analysis of sputum conversion revealed low sensitivity and modest specificity of this measure for the prediction of treatment failure in individuals except when used in large clinical trials (41). Chest radiographs are commonly used to assess response but are not universally available, and assessment is difficult to standardize (42). The lack of effective treatment monitoring can lead to the development and spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB (42), which are mainly attributed to nonadherence or to inappropriate drug regimens. This risk has a detrimental impact on global TB control and impairs monitoring of the treatment efficacy of badly needed new drugs.

Latent Tuberculosis

As discussed above, the global prevalence of M. tuberculosis infection is about 32% (6). However, most infected individuals are asymptomatic (i.e., they have latent TB) and have no clinical evidence of disease. It is thought that latent individuals maintain the infection in a quiescent form. Epidemiological studies carried out in both developing and developed countries indicate that 5–10% of latent individuals will develop active TB during their lifetime, with the highest risk following infection in early adulthood and the lifetime risk declining each year after infection (7, 8). However, this risk is substantially higher in individuals who are immunosuppressed, particularly those with HIV coinfection (43). This latent state is thought to be maintained by an active immune response in the host initiated by the infecting M. tuberculosis bacilli, permitting host-controlled persistence of the organism (44). Molecular epidemiological evidence suggests that the original infecting strain can lead to reactivation of TB up to 30 years after the initial infection (45). This hypothesis is in keeping with previous reports that live and viable M. tuberculosis bacilli can be recovered from incidental TB lesions discovered postmortem in individuals who died of other causes (46) and from postmortem lesions of latent individuals (47). Thus, infection with M. tuberculosis can result in two extremely diverse clinical phenotypes: symptomatic active TB disease, comprising systemic features such as fever and weight loss, with localized symptoms of tissue destruction at the site of active infection and with actively replicating transmissible bacteria; and the asymptomatic latent state. More recently, studies have recognized that the heterogeneity of the host response to M. tuberculosis infection extends beyond the two extremes of active and latent TB in that each encompasses a heterogeneous group of clinical states (18, 48, 49) (Figure 2).

In latent TB, M. tuberculosis infection or exposure can be shown only by demonstrating the host’s reactivity to M. tuberculosis antigens, classically using the tuberculin skin test (TST) (50). The patient is intradermally challenged with an extract containing M. tuberculosis antigens, originally tuberculin, a glycerine extract of M. tuberculosis (51), but now replaced with a commercially purified protein derivative (PPD) (52). The resulting induration in the skin, which is due to the development of a delayed-type hypersensitivity reaction, is measured in millimeters. In latent infection, the TST is more frequently negative in those individuals most at risk of progression to active disease: the young, the elderly, and the immunosuppressed (53).
Heterogeneity resulting from *M. tuberculosis* infection. It has long been recognized that infection with *M. tuberculosis* can result in two extremely diverse clinical phenotypes: symptomatic active tuberculosis disease, and the asymptomatic latent state, as shown in Figure 1. However, there is increasing recognition that this latent state represents diverse responses to infection and, consequently, heterogeneous clinical outcomes. In latent individuals, *M. tuberculosis* infection or exposure is inferred by demonstrating the host’s reactivity to mycobacterial antigens using either the classic tuberculin skin test (TST) (50) or the more recent IFN-γ release assays (IGRAs), which show reactivity to *M. tuberculosis*-specific antigens (58) via the production of IFN-γ by blood cells. However, these crude immunological responses and the related positive TST and/or IGRA results are shared by individuals who have cleared infection and developed a detectable adaptive immune response; those who have mounted an adaptive immune response, yet who remain infected but asymptomatic; and those with subclinical or established active disease. Conversely, these responses may not be present in individuals exposed to *M. tuberculosis* for whom the exposure was insufficient to lead to infection or in those who may have cleared infection without developing a detectable adaptive immune response, with consequent negative TST and/or IGRA results. Equally, infected individuals in whom the anti-tuberculosis immune response is highly localized or is of insufficient magnitude to be detected systemically may also have negative test results. Thus, the true spectrum of responses to *M. tuberculosis* infection is broader and more heterogeneous than previously supposed.

In addition, because PPD is prepared from culture filtrate of *M. tuberculosis*, it contains over 200 antigens also found in the attenuated *M. bovis* BCG vaccine and in many environmental nontuberculous mycobacteria (54), and it therefore has limited specificity. Thus, false-positive TST reactions can occur both in those who have been vaccinated, which accounts for more than 3 billion people worldwide (55), and in those who have been sensitized to these common antigens through exposure to environmental nontuberculous mycobacteria (56, 57).

More recently, assays have been developed that utilize more specific *M. tuberculosis* antigens [predominantly early secretory antigen target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10)] that are absent from BCG and most nontuberculous mycobacteria (58). Reactivity to these *M. tuberculosis* antigens is assessed in terms of production of IFN-γ by blood cells using IFN-γ release assays (IGRAs), measured either by enzyme-linked immunosay after whole blood incubation (Qiagen) or by the enzyme-linked immunospot technique, which requires isolation of peripheral blood
mononuclear cells (PBMCs) before incubation (TSPOT.TB™, Oxford Immunotec, Oxford, UK). Although these tests offer improved specificity, along with possibly improved sensitivity (54), no test is currently available to differentiate latent from active TB disease. Furthermore, there is no test to identify those latent individuals who may progress to active TB or those who have subclinical disease. The ability to identify those latent individuals who are most at risk of reactivation would help target preventative therapy; such targeting is important because drug treatment is lengthy and potentially toxic.

HETEROGENEITY OF LATENT TUBERCULOSIS

Numerous studies have observed that only between 20% and 50% of latent close contacts of highly infectious TB cases develop a positive TST skin reaction after exposure, whereas 1–2% of these close contacts may eventually develop active TB (59–61) (Figure 2). Taken together, these data suggest that TST non-responders in this situation may represent individuals who were not infected or those who were resistant to M. tuberculosis infection and may have cleared infection through an effective innate immune response (13), although either case is difficult to prove in humans. Alternatively, such a low-grade infection with M. tuberculosis may result in a contained and localized immune response in the lung, whether innate or adaptive, that is not detectable by the TST or an IGRA. In addition, it is unclear whether the M. tuberculosis bacilli are actually eliminated or just kept under tight immune control. An intriguing study was performed in a highly endemic area in South Africa, where, despite the high TB-exposure rate, 20% of the exposed population remain TST negative. This study demonstrated that the TST negativity and blood cell production of IFN-γ and TNF in response to M. tuberculosis antigens were under strict genetic control, suggesting T cell–independent resistance (62, 63). Studies have also suggested that the subsequent risk of developing disease in close contacts of TB patients was greatest among reactors who were initially most sensitive to tuberculin (7). This risk variance could be reflective of the genetic background of the individual but possibly also of the dose of the challenging M. tuberculosis infection. Latent TB is defined not by the confirmed presence of M. tuberculosis but rather by the presence of an immune response directed against M. tuberculosis antigens. Thus, latent TB is reflective of a heterogeneous group of individuals: those who have subclinical disease (18); those who will progress to primary active disease; those who maintain persistent, lifelong infection; those who temporarily suppress infection but later succumb and develop active disease, possibly as a result of immunosuppression or some other event (i.e., true latent infection); and those who are able—either through innate or adaptive immunity or the combination—to effectively clear the pathogen (Figure 2). However, it is also possible that all individuals exposed sufficiently to be infected remain so without ever clearing the pathogen.

Both humans and nonhuman primates infected with M. tuberculosis show heterogeneity of lung lesion types (18, 64). The heterogeneity of latent and active TB has been elegantly demonstrated in the cynomolgus macaque model of TB (65). In this model, macaques were directly infected by bronchoscopic instillation of low doses of virulent M. tuberculosis into the lung, and all monkeys were successfully infected. Thus, the time and nature of the initial infection can be completely ascertained, as opposed to the situation when studying human patients who are exposed to coughs of infected transmitting TB patients via droplets that contain varying numbers of M. tuberculosis bacilli (66).

The cynomolgus macaque model of TB is potentially the most useful for the study of latent TB, given that the authors observed diverse outcomes in response to identical experimental infection. The infection resulted in pathological presentations ranging from sterile tissue, to caseous hypoxic lesions containing variable numbers of bacilli, to liquefied cavities with a
very high load of replicating bacilli (65). Active chronic infection was observed in 50–60% of monkeys, was characterized by clear signs of infection or disease on serial thoracic radiographs and in other tests, and was typified by eventual progression to full disease. Specifically, the outcomes included macaques that progressed rapidly and succumbed to active disease, others that developed active disease over a more chronic course (including one who spontaneously resolved the infection), and those that displayed no evidence of disease even though they were clearly infected and had clinical characteristics similar to latent TB in humans. One of these monkeys with latent characteristics later developed active disease (65). The ratio of latent to active disease is somewhat reversed in this model, with most animals developing active disease, but this presumably represents the potentially larger infecting dose and the direct route of administration used compared with the more passive exposure of human patients. If researchers could combine this model with the methods used in the equally innovative guinea pig model to assess the infectiousness of expired air from TB patients (whereby the exhausted air from the isolation rooms of patients with active TB is circulated through the experimental facility), the technique might recreate a more natural exposure (67). Nonetheless, the cynomolgus macaque model recapitulates many of the major responses to M. tuberculosis infection observed in humans.

The heterogeneity within the latent TB population is gaining more widespread acceptance (18) and highlights the need for diagnostic tools that differentiate the full spectrum of these diverse responses to infection (18, 49, 68). Moreover, the outcome of infection with M. tuberculosis and whether individuals control the infection or go on to develop active TB is complex and to a large extent determined by variations in the host and the pathogen that are still poorly understood (2). Although some of the immune factors controlling M. tuberculosis infection that prevent the development of active TB have been defined (reviewed in 11–15, 17), the host molecular determinants distinguishing latent and active TB are undefined, and host factors underlying the development of active TB disease are as yet unclear.

GLOBAL ANALYSIS OF HUMAN TUBERCULOSIS

Transcriptomics Advances Our Knowledge of Human Disease

Over the past decade, transcriptional profiling has been successfully applied to human disease both to improve our understanding of the underlying molecular processes contributing to pathogenesis and to improve patient classification by providing surrogate markers of clinical phenotyping. This process has been most proficiently demonstrated in the study of cancers, such as in studies of bone marrow cells of patients with acute myeloid leukemia, acute lymphoblastic leukemia (69), and breast cancer, where transcriptional signatures are used to accurately predict prognosis and effectively direct treatment (70). In recent years, transcriptional analysis has been applied to whole blood, which offers an easily accessible source that has the capacity to reflect global immunological and pathological host changes. Studies of patients with autoimmune diseases have led to the novel identification of candidate molecules and pathways underlying human disease, leading to prospective new therapeutic targets and to potential diagnostic and prognostic biomarkers (71).

Blood Transcriptional Profiling Reveals a Signature of Active Tuberculosis

Using an unbiased, comprehensive, whole-genome microarray study of whole blood from patients with active and latent TB, and healthy controls, we have gained an understanding of the immune response and potential factors that lead to the pathogenesis of TB disease (72). Using unsupervised analysis followed by statistical filtering, we first established a distinct 393-transcript signature, present in the blood...
of patients with active TB recruited in London, that was absent in most latent individuals and healthy controls. The signature of active TB was validated in a further set of samples from patients and controls recruited in London and a setting of high disease prevalence, South Africa. Complementary analytical approaches at the module, pathway, and gene levels allowed us to identify a striking IFN-inducible signature of active TB and a previously underappreciated association with type I IFN-inducible genes and disease susceptibility (Figure 3) (72). This IFN-inducible signature significantly correlated with the extent of lung radiographic disease and disappeared after 2 months of successful treatment (Figure 3).

We have since determined that a transcriptional change toward that of healthy controls occurs as early as 2 weeks after treatment initiation (73). These findings offer promise for much needed improvement in pulmonary TB treatment monitoring by using an early change in the blood transcriptional signature. Currently, treatment monitoring is available only after 2 months of treatment, by monitoring for sputum clearance of the bacilli, and, as discussed above, sputum diagnosis is not feasible in 30% to 50% of individuals with active TB. Importantly, these potential early biomarkers of treatment response could also enhance the evaluation of new drugs. Perhaps unexpectedly, we also found, on examining the different cell populations present in the whole blood of active TB patients versus controls, that the IFN-inducible genes were predominantly expressed in neutrophils and to some extent in monocytes, but not in T cells (72). This finding suggested that overactivation of neutrophils by IFNs during infection may contribute to disease pathogenesis in TB. It is unclear where in the body these neutrophils receive the signal to activate this set of genes from IFNs. In addition to this altered cytokine gene expression in discrete cells, the transcriptional signature reflected changes in cellular composition, with B and T cell genes being underrepresented in the whole blood (72). This reduction in B and T cell numbers was shown (by flow cytometry and analysis of gene expression in purified cells) to be attributable to reduced cell numbers in the blood, which could result from apoptosis or the migration of cells to the infected tissue. Thus, blood transcriptional profiling of TB disease can highlight immune factors that potentially play a role in disease. Our transcriptional blood signature was independently validated in additional studies (74–77); a significant percentage of the differentially expressed genes present in the blood of active TB patients in our study were also found to be present in active TB patients from other cohorts in Africa and Asia (75–77). A further study that looked specifically at T cells in active TB patients compared with latent TB patients identified upregulation of particular discriminating immunoregulatory genes, including JAK3, SOCS3, and IL2RA (78).

In addition, our study revealed that although the 393-transcript signature of active TB was absent in most latent individuals, it was present...
in 10–20% of them (72), demonstrating molecular heterogeneity of latent TB, which is consistent with the concept of a spectrum of responses to *M. tuberculosis* infection in latency. Whether this molecular heterogeneity reflects divergent clinical outcomes requires further work.

**Comparison of the Blood Transcriptional Signature of Active Tuberculosis With Those of Other Diseases**

Our study also compared the transcriptional signature of active TB patients to patients...
with other diseases, including infectious and autoimmune diseases (72). Using a statistical approach termed “analysis of significance” (79) and a modular data-mining strategy of the blood transcriptome (80), we were able to distinguish patients with active TB from those with streptococcal and staphylococcal infections and autoimmune diseases (72).

Whole blood transcriptional studies have also been used to compare active TB with the analogous respiratory disease sarcoidosis (81, 82). Sarcoidosis is a multi-system granulomatous disease of unknown etiology that affects individuals worldwide and is predominantly a respiratory disorder presenting with very similar clinical, histological, and radiological features to active TB (83). Interestingly, two published studies and a larger study from our own laboratory (C.I. Bloom, A. O’Garra, unpublished observations) comparing active TB to sarcoidosis all found significant overlap in the differentially expressed genes, including the IFN-inducible genes (81, 82). This overlap most likely reflects the similar underlying immune mechanisms of both granulomatous diseases, although some transcripts are apparently unique to each disease. We also found that the active TB and sarcoidosis blood signatures differed from signatures present in patients with lung cancer and community-acquired pneumonias, pointing to the differing host factors at play in these pulmonary diseases, despite their sharing many common histopathological and radiological characteristics (C.I. Bloom, A. O’Garra, unpublished observations). Examination of immune responses using a global systems approach has been a powerful tool for improving our understanding of the molecular heterogeneity of latent and active TB and has revealed a previously unappreciated potential role of type I IFN in the development of active TB in humans (72). However, to dissect the different stages of M. tuberculosis infection and disease, experimental models are essential.

HUMAN TUBERCULOSIS AND EXPERIMENTAL ANIMAL MODELS

As discussed above and previously reviewed (18), the pathology of human TB suggests that the disease is heterogeneous, consisting of a continuum of lesions (47, 64) that reflect stages of latent and active TB (18). Although the mouse model offers the best tools for the study of the immune response to pathogens, TB disease in commonly used mouse strains, such as C57BL/6 or BALB/c mice, does not readily recapitulate the human pathology seen in the human disease (20, 21, 84). The granulomas are poorly organized and exclusively cellular; they lack fibrosis or hypoxia (85); the bacterial counts remain at a relatively high but apparently controlled level throughout the course of the disease; and all the mice ultimately die of progressive infection. Thus, the model lacks the range of latency to active disease seen in humans (18). However, although we lack a good model of latency in mice, TB disease in guinea pigs, rabbits, and mice resulting from aerosol infection with low-dose virulent M. tuberculosis exhibits many of the important features of human TB (86). Moreover, the use of inbred mice with genetic deletions in genes encoding IL-12, IFN-γ (11, 12, 87, 88), and TNF-α (16), as well as of mice depleted of CD4+ T cells (11, 13, 89), shows that these immune factors are critical for controlling M. tuberculosis infection in the mouse. These findings in mouse models have been validated in human disease studies where TNF-α (17), IL-12, and IFN-γ (14, 15) have been shown to be critical for preventing TB in humans, as are CD4+ T cells, without which HIV-infected individuals succumb rapidly to TB disease (90, 91). Host immune factors controlling M. tuberculosis infection are discussed in more detail later in this review; we emphasize here the similarities in host-protective factors in both mouse models and human TB. Furthermore, there are alternative strains of mice, including the CBA/J, DBA/2, and C3H, that are highly susceptible to M. tuberculosis.
infection, and the pathology reported in their lungs more closely resembles lesions seen in human TB disease (20, 21, 84, 92–94). Such intrinsically susceptible mouse strains are a potentially powerful tool for uncovering mechanisms underlying the pathogenesis of TB, and this tool may be further improved by using clinical isolates of *M. tuberculosis*, which may lead to TB disease that even more closely resembles that in humans. Furthermore, these susceptible mouse strains may reveal important factors that contribute to the development of active TB, as has been shown for the contribution of neutrophils (95), and are discussed in more detail below.

A recently described rabbit model of latent TB is characterized by persistent but controlled infection that can be reactivated upon immunosuppression (96). The rabbit model has been further developed as a model of cavitary TB, which reflects many aspects of the human disease, such as similar lung histopathology, the development of caseation and lung cavities, and chronic progressive granulomatous pulmonary disease, following aerosol infection with the Beijing lineage HN878 *M. tuberculosis* strain (97, 98). This model was associated with delayed and suboptimal macrophage activation and delayed differentiation and accumulation of antigen-specific T cells (98), demonstrating gene expression that reflects IFN-γ, IL-4, and B cell activation, as shown by lung transcriptomics. Thus, an interesting line of inquiry is to determine the blood transcriptome in this cavitary model of TB and whether type I IFN–inducible genes are induced, as would be anticipated from studies with HN878 in the mouse model from the same group (24, 99) and from our own studies of the type I and type II IFN–inducible signature in humans with active TB (72).

As discussed above, infection of nonhuman primates with low doses of *M. tuberculosis* results in a spectrum of disease and pathology very similar to that seen in humans, providing invaluable information and systems to uncover the mechanisms of control or disease progression in TB (65, 100). However, although essential to pursue, this model is costly, resource intensive, and limited by a lack of host genetic variants and tools for studying the immune response that are available in the mouse. Hence, TB research must maintain all the different animal models and compare and contrast them with human disease, as well as with each other, to maximize our mechanistic knowledge of the host-pathogen interactions that influence the outcome of disease.

### DIFFERENT STAGES AND FACTORS IN THE IMMUNE RESPONSE IN TUBERCULOSIS

#### Orchestration of the Host Immune Response to *M. tuberculosis* Results in the Formation of Granulomas

The role of granulomas is not always clear: Are they purely protective for the host or do they promote infection? Do they contribute to tissue pathology? Granulomas likely contribute to all these, depending on the stage of disease, whether the *M. tuberculosis* bacilli are being controlled by innate and/or adaptive immune responses, or whether the disease has progressed to active TB (18).

TB granulomas have been studied for over a century and reveal the pattern of immune responses that occur at the different stages of the disease. Heterogeneity in granuloma morphology was discovered in human postmortem studies more than 50 years ago, even in lesions of only 1 mm³, in patients considered to have minimal pulmonary TB, and in those who did not die from their disease (64). Human TB granulomas are composed of a central mass of infected macrophages, stimulated macrophages that have differentiated into multinucleated giant cells, epithelioid cells and foamy macrophages loaded with lipid droplets, and neutrophils (101). This inner accumulation of cells becomes surrounded by lymphocytes, largely CD4⁺ T cells but also CD8⁺ T cells and B cells, and by fibroblasts, which create a peripheral fibrotic capsule (102), although T cells appear to have limited...
antigen-presenting cell function in the granuloma (103). Various proinflammatory and inhibitory cytokines and chemokines, in addition to adhesion molecules, play key roles in the formation of granulomas (reviewed in 102). A study of lung tissue specimens from patients with MDR TB found that the formation of granulomas required a minimal size of 0.1 mm³ and showed the presence of lymphoid follicle-like structures in the peripheral margins of the granulomas, composed predominantly of B cells and some CD4⁺ and CD8⁺ T cells, surrounding infected macrophages (104). The authors concluded that mycobacteria can survive within the granulomas, in the periphery of the granulomas, and even further afield in apparently normal, healthy parenchymal tissues (104, 105).

One of the classical features of human TB granulomas is the presence of a necrotic caseous core that is thought to be secondary to cell lysis and that results in a central hypoxic, hostile environment (106). Such hypoxic granulomas have also been reported in guinea pigs and nonhuman primates (85) but not in the standard C57BL/6 or BALB/c mice infected with strains of M. tuberculosis Erdman (106) or H37Rv or the hypervirulent clinical isolate HN878 (85). Recently, Reece et al. (107) showed that Nos2-deficient mice control M. tuberculosis infection in hypoxic lung granulomas by the action of serine proteases. In addition, a lung fibrotic response has been reported to distinguish resistance and susceptibility during pulmonary infection with M. tuberculosis, with the susceptible DBA/2 strain showing fibrotic lesions with exudative, necrotic alveolitis and the presence of degenerative neutrophils containing bacilli (93). With respect to the hypoxic granuloma, investigators have proposed that in latent TB the bacilli reside in the central hypoxic zone in a metabolically altered state, but that in active TB they can replicate in peripheral oxygenated areas (18). However, as discussed above, the distinction may not be that clear cut (104, 105). Another question is whether the granulomas always protect against M. tuberculosis infection; indeed, it has been suggested that the pathogen may be able to engineer a supportive environment in the granuloma through, for example, the manipulation of macrophage lipid metabolism (101).

The guinea pig is thought to provide the small animal model that most closely resembles the immunopathological response found in humans infected with M. tuberculosis (86, 108). The first phase of the primary pulmonary lesion in guinea pigs is the influx of granulocytes and eosinophils, after which numerous macrophages and lymphocytes, along with fewer granulocytes, coalesce to form the classical tuberculous granuloma, before further expansion into the lung parenchyma and the formation of a central necrotic focus (108).

The nonhuman primate model has provided valuable information with respect to the spectrum of disease encompassing the continuum of latent and active TB (100), as discussed above. Lung histology from cynomolgus macaques presenting with active TB after intratracheal infection with M. tuberculosis has revealed various granuloma types not only between the macaques but also within each organ (100). Three main types have been described in active TB: the classical caseous granuloma, with central eosinophilic debris surrounded by macrophages and a layer of lymphocytes; the non-necrotizing granuloma, with an internal compact core of macrophages and some neutrophils surrounded by a lymphocyte layer; and the suppurative granuloma, with a central core of degenerative neutrophils surrounded by macrophages and multinucleated giant cells and an outer envelope of lymphocytes (100).

Using an elegant model of zebrafish infected with Mycobacterium marinum to mimic TB, Ramakrishnan and colleagues (109, 110) showed that virulent intracellular mycobacteria induce recruitment of macrophages to early granulomas and that these macrophages are highly motile, leaving the granuloma after becoming infected. This suggests that the mycobacteria are using the host to facilitate the spread of infection (109, 110). Although zebrafish do not have lungs or an adaptive immune system, they provide a system for dissecting the
very early response to mycobacterial infection, which has to date revealed mechanisms relevant to *M. tuberculosis* infection in humans (111) (discussed in more detail below). Such mechanisms may also be relevant to the response seen in patients who lack an adequate adaptive immune response, such as HIV-infected individuals.

**Initial Events Following *M. tuberculosis* Infection**

*M. tuberculosis* is spread by airborne droplet nuclei, transmitted when an infected individual coughs and disperses these droplets, which can then be inhaled into the airways and alveoli of a new host (66). Still unclear is the exact dose of transmitting *M. tuberculosis* that results in infection or not and/or active disease or not, as well as the status of the *M. tuberculosis* bacilli in those droplets (112). Experimental models have shown that the early host response to *M. tuberculosis* infection is characterized by an influx of phagocytic cells including primarily resident alveolar macrophages and recruited neutrophils (113). Following the establishment of *M. tuberculosis* infection in the airways and lung parenchyma, the bacilli are believed to be phagocytosed by the alveolar macrophages (113) and are taken up by neutrophils (114, 115) and dendritic cells (DCs) (116) (Figure 4). Macrophages and neutrophils may constitute a first line of defense (117) by, for example, expression of antimicrobial peptides that may function in the early immune response (118–122). Appropriate macrophage and neutrophil activation to restrict and/or kill the pathogen is undoubtedly also determined by extrinsic innate and adaptive immune factors that together play a critical role in determining the outcome of the immune response to *M. tuberculosis* infection (119, 123, 124). After infection of the host with *M. tuberculosis*, macrophages and neutrophils and the context of their activation may influence the subsequent immune response toward potential clearance or containment of the pathogen, resulting in persistent latent infection or the development of active disease (27).

Entry of *M. tuberculosis* into the macrophage is mediated by a diverse array of receptors, including scavenger receptors, complement receptors, and the mannose receptor (reviewed extensively in, e.g., 125). Experiments using murine peritoneal macrophages established that once *M. tuberculosis* is internalized, a sequence of events results in the creation of the phagosome around the phagocytosed bacillus (126). The fate of intracellular bacteria such as *M. tuberculosis* can be influenced by autophagy, a process whereby components of the cytoplasm, including organelles and intracellular pathogens, are sequestered in an autophagosome and delivered to the lysosome for degradation (127, 128). Activation of autophagy, by IFN-γ for example, results in phagosome maturation and an increase in its acidification and *M. tuberculosis* killing (127, 128). However, in contrast to nonviable bacilli (126), viable and virulent *M. tuberculosis* bacilli are able to prevent phagolysosomal fusion and persist in the phagosome, preventing acidification of the phagosomal compartment (129), thus adapting to the intracellular environment of the macrophage and creating a niche for survival. Opsonization of the bacilli prior to infection inhibits this blockade of phagolysosomal fusion (130). Macrophages can eliminate mycobacteria via different mechanisms (131) if appropriately activated (discussed later in this review). Owing to the wealth of genetic and immunological tools available, the mouse model has been invaluable in delineating early events after aerosol infection with *M. tuberculosis* and the stages of cellular and molecular innate and adaptive immune responses contributing to protection against or the development of disease (11–13, 132, 133). However, it is critical to compare data between different experimental models and human disease whenever possible to understand the factors that determine protection or pathogenesis in TB.

Dissemination of *M. tuberculosis* in the mouse precedes the initiation of T cell immunity in the lung-draining mediastinal lymph...
The cellular immune response to *M. tuberculosis*. Following aerosol infection with *M. tuberculosis*, resident lung alveolar macrophages (1a), neutrophils (1b) and lung DCs (1c) can become infected, leading to the production and secretion of antimicrobial peptides, cytokines, and chemokines. The balance of lipid mediators, such as prostaglandin E2 (proapoptotic) or lipoxin (LX) A4 (pronecrotic), within infected macrophages plays a major role in determining downstream pathways leading to the induction of either apoptosis or necrosis. Infected apoptotic cells can be taken up by resident lung DCs or efferocytosed by uninfected lung macrophages (1c). *M. tuberculosis*-infected DCs migrate to the local lung-draining lymph nodes by 8–12 days post infection. DCs migrate to the lymph nodes under the influence of IL-12(p40)2 and IL-12p70 and that of the chemokines CCL19 and CCL21 (2), to drive naive T cell differentiation toward a Th1 phenotype (3). Protective antigen-specific Th1 cells migrate back to the lungs in a chemokine-dependent manner 14–17 days after the point of initial infection/exposure (4) and produce IFN-γ, leading to macrophage activation, cytokine production, the induction of microbicidal factors including iNOS (5), and bacterial control.

Dissemination of *M. tuberculosis* may aid in the initiation of an appropriate and timely adaptive immune response, although this response may be under strict host genetic control (134).
Macrophage Apoptosis as a Defense Against M. tuberculosis Infection

Infection of macrophages with M. tuberculosis can induce necrotic death, defined by cell lysis, which allows exit from macrophages and therefore cell-to-cell spread of the bacilli. Alternatively, infection can result in apoptotic death of the macrophages that maintain an intact plasma membrane (123) (Figure 4) and is associated with diminished pathogen viability and enhanced immunity (123). A role for apoptosis as an antimycobacterial mechanism was first reported in human alveolar macrophages where attenuated mycobacterial strains, including M. tuberculosis H37Ra, exhibited reduced viability as their host macrophages underwent apoptosis (135). More recently, this work has been extended to show contact-dependent apoptosis of bystander macrophages after infection with M. tuberculosis H37Ra, suggesting another mechanism by which bacterial spread is limited by the host (136). In contrast, virulent strains of M. tuberculosis induce little macrophage apoptosis and grow intracellularly and progressively in these cells (136). That inhibition of apoptosis is a virulence mechanism of M. tuberculosis has been validated in vivo by demonstration of attenuation of the proapoptotic M. tuberculosis secA2 and nuoG deletion mutants upon infection (123, 137, 138). Inactivation of the secA2 gene in M. tuberculosis, which encodes a component of a virulence-associated protein secretion system, enhanced apoptosis of infected macrophages by diminishing the secretion of mycobacterial superoxide dismutase (137). Deletion of secA2 markedly increased priming of antigen-specific CD8+ T cells in vivo, and vaccination of mice and guinea pigs with a secA2 mutant significantly increased CD4+ T cell responses and resistance to M. tuberculosis challenge (137).

Building on this, Behar, Remold, and colleagues (139) have recently demonstrated that M. tuberculosis—infected macrophages are themselves rapidly engulfed by uninfected macrophages through a process called efferocytosis (140) (Figure 4), generally regarded as a constitutive housekeeping function of macrophages. Engulfment of M. tuberculosis sequestered within an apoptotic macrophage further compartmentalizes the bacilli, delivering them together with apoptotic debris to the lysosomal compartment—efferocytosis—which is followed by killing of the M. tuberculosis bacilli (19).

The type of cell death that is induced following M. tuberculosis infection is regulated by the lipid mediators eicosanoids, prostaglandin E2 (PGE2) (proapoptotic), and lipoxin A4 (LXA4) (pronecrotic), and this regulation plays a major role in determining the outcome of infection (123, 141–143). This is discussed in greater detail below.

Virulent strains of M. tuberculosis evade innate defense mechanisms of the host by inducing LXA4 and inhibiting PGE2 production, leading to macrophage necrosis and inhibition of macrophage apoptosis, ultimately resulting in mycobacterial spread (123, 142, 143) (Figure 4). Macrophages from mice deficient in 5-lipoxygenase (Alox5−/− mice), which cannot synthesize LXA4, undergo more apoptosis after infection with virulent M. tuberculosis, and macrophages from mice that lack prostaglandin E synthase (Ptges−/− mice), which cannot produce PGE2, undergo more necrosis after infection even with avirulent strains and are more susceptible to aerosol infection with virulent M. tuberculosis (142). Alox5−/− mice may be more resistant, and Ptges−/− mice more susceptible, because, by activation of the 5-lipoxygenase pathway, M. tuberculosis infection not only inhibits macrophage apoptosis, but also prevents cross-presentation of M. tuberculosis antigens by DCs, thus impeding the initiation of T cell immunity (141, 143) (discussed in more detail below).

Roles of the Neutrophil in the Immune Response to M. tuberculosis: Friend and Foe

Neutrophils are infected with mycobacteria in human TB, a finding that agrees with
experimental models suggesting a role for these cells as permissive hosts (115, 144, 145). Although granulocytes may play a role in granuloma formation in relatively resistant mice (146), many reports support a negative role for neutrophils/granulocytes in TB pathogenesis in genetically susceptible mouse strains (95, 114) and in active TB patients, where respiratory failure and mortality are associated with increased blood neutrophil levels (147, 148). Susceptible mouse strains show high numbers of neutrophils (95, 114), with accelerated recruitment into the lungs (95). Furthermore, neutrophil elimination leads to enhanced protection in susceptible mice (95, 114). Collectively, these reports suggest a detrimental role for neutrophils in the pathogenesis of TB.

However, lung neutrophils facilitate activation of naive antigen-specific CD4$^+$ T cells during M. tuberculosis infection and promote an anti-M. tuberculosis adaptive immune response by delivering the bacilli to DCs in a form that makes DCs more effective initiators of CD4$^+$ T cell activation (149) (Figure 4). However, as discussed above for macrophages, virulent M. tuberculosis H37Rv inhibits apoptosis of neutrophils (150), and this leads to delayed activation of naive CD4$^+$ T cells in the lung-draining lymph nodes (150). The proapoptotic M. tuberculosis nuoG mutant resulted in fewer bacteria per infected neutrophil, accelerated bacterial acquisition by DCs, earlier trafficking of these DCs to lymph nodes, and faster CD4$^+$ T cell priming (150). In this case, neutrophil depletion abrogated accelerated CD4$^+$ and CD8$^+$ T cell priming by the nuoG mutant, suggesting that inhibition of neutrophil apoptosis by virulent M. tuberculosis delays and impairs the induction of T cell immunity early in the course of infection (150).

Thus, whether neutrophils have a protective or detrimental effect during an immune response to M. tuberculosis infection (Figure 4) may be determined by the genetics of the pathogen as well as by the genetics of the host and the stage of TB disease. The tissue environment and network of cytokines induced will undoubtedly also affect the neutrophil and the subsequent immune response elicited toward M. tuberculosis infection. For example, neutrophils are dominant producers of IL-10 in the lung (151). Depletion of neutrophils reduces the lung bacterial load while enhancing IL-6 and IL-17, but not IFN-γ, responses (151). Data from our own lab suggest, however, that IL-10 production by different immune cells during M. tuberculosis infection depends on both the mycobacterial strain and the stage after M. tuberculosis infection (P.S. Redford, A. O’Garra, unpublished data). Also, as we discuss below, IL-17 may contribute to enhanced neutrophil-mediated disease during M. tuberculosis infection (152, 153). Tight control of neutrophil function and number, during an immune response to M. tuberculosis infection, would thus allow these cells to provide a protective role without contributing to pathology and exacerbation of disease. In keeping with this, a recent finding has demonstrated that IFN-γ inhibits CD4$^+$ T cell production of IL-17, impairing both neutrophil survival and the accumulation of pathogenic neutrophils in the infected lung, and thereby contributing to decreased lung inflammation and improved disease outcome (154). Neutrophilia during TB may thus indicate failed Th1 immunity or loss of IFN-γ responsiveness (154). Excessive signaling of type I IFN in neutrophils and macrophages may also contribute to neutrophilia, as we have demonstrated in human active TB (72). More recently, we found that type I IFN signaling results in a loss of IFN-γ responsiveness (F. McNab, J. Ewbank, A. O’Garra, unpublished observations). Hence, macrophages and neutrophils play key roles in protection against M. tuberculosis infection by their direct antimicrobial activities and their subsequent ability to help shape the activation of the adaptive immune response. However, such roles may be manipulated by M. tuberculosis itself and by induction of distinct intrinsic and extrinsic host factors that may contribute to the development of a protective response or pathogenesis and disease.
INNATE FACTORS

TNF Is a Key Factor in Protection Against M. tuberculosis Infection, But How?

Studies using mice treated with anti-TNF antibodies or mice in which the 55-kDa TNF receptor gene was disrupted revealed that TNF was essential for the control of M. tuberculosis infection (12, 16). M. tuberculosis was lethal in both the antibody-treated and the genetically deficient mice, with increased bacillary load and necrosis within granulomas, which showed a marked qualitative difference (16). TNF−/− mice also showed increased susceptibility to M. tuberculosis infection (155, 156). Neutralization of TNF in mice in a low-dose M. tuberculosis infection model of persistent TB resulted in a fatal reactivation of infection, demonstrating a role for TNF in the containment of persistent TB (157–159). This reactivation of infection was accompanied by severe pulmonary histopathological deterioration, a reduction in iNOS activation, and an increase in IL-10 expression, but the IL-12p40 and IFN-γ responses were normal (159). The critical role of TNF in control of M. tuberculosis infection in humans was illustrated by the increased rate of reactivation of active TB in subjects with latent TB who received anti-TNF therapy for rheumatoid arthritis or Crohn’s disease (17, 160). A more than five-fold increase in the rate of TB among patients receiving treatment was observed, with a quarter of patients developing disseminated disease and a further third having localized extrapulmonary disease, suggesting that these cases predominantly represent reactivation of previously controlled M. tuberculosis infection (17, 160).

TNF can be produced by multiple immune cells, including macrophages, neutrophils, DCs, and T cells (161), and has multiple functions (162). In addition to TNF’s multiple roles in activating macrophages and inducing chemokine production (12), investigators have suggested that it is required for the formation and maintenance of the integrity of the granuloma (155, 158, 163). This role was offered as the explanation for the reactivation of TB seen upon administration of monoclonal antibodies against TNF (157–159) and was consistent with the theory that granulomas benefited the host by containing and controlling M. tuberculosis (12). However, work using the mouse model of M. tuberculosis infection showed that granuloma formation could occur even in the absence of TNF signaling, although these granulomas were delayed and were more necrotic, with higher bacillary numbers (16). Moreover, more recent reports of patients developing TB after anti-TNF treatment found that biopsies of these patients displayed classical granuloma structures (164). Using the cynomolgus macaque model, Lin et al. (165) demonstrated that TNF neutralization during M. tuberculosis infection results in fulminant and disseminated disease and causes reactivation in most animals with latent TB, as determined by gross pathological examination and bacterial burden. Interestingly, these researchers noted a spectrum of dissemination, including extrapulmonary disease, although monkeys that developed primary and reactivation TB after anti-TNF treatment had similar granuloma structure and composition to that of control monkeys with active disease (165). This finding is in keeping with studies using the Mycobacterium marinum–infected zebrafish model, which demonstrated that in the absence of TNF, not only intracellular bacterial growth but also granuloma formation was accelerated and followed by necrotic death of overladen macrophages and granuloma breakdown (166). This is in line with other reports suggesting that the major role of TNF is in boosting the intracellular killing of bacilli (167) and not in the formation of the tuberculous granuloma (rather, TNF is pivotal in the tuberculous granuloma’s maintenance) (166). Using the same zebrafish model, this group has also presented convincing data that—in the early stages of this mycobacterial infection in the absence of an established adaptive immune response—the granuloma may in fact enhance the dissemination of mycobacteria by recruiting uninfected macrophages to the site of infection, which then phagocytose infected...
macrophages and thus become infected themselves. These secondarily infected macrophages may then leave the primary granuloma and initiate the formation of secondary granulomas, both at local tissue sites through migration and at distant sites through hematogenous spread (109). This zebrafish model may prove to be a blueprint for events occurring prior to the development of the adaptive immune response or in the event of a disruption of adaptive immunity, for example during HIV infection.

**Does Too Little or Too Much Inflammation or the Kind of Inflammation Contribute to Disease Resistance or Susceptibility in Tuberculosis?**

**Eicosanoids can control or promote *M. tuberculosis* infection.** As discussed above, the balance between the eicosanoids, PGE2 (which promotes apoptosis of innate immune cells such as macrophages infected with *M. tuberculosis*), and LXA4 (which in contrast promotes their necrosis) plays a major role in determining the outcome of *M. tuberculosis* infection, and this balance is determined by the virulence of the infecting *M. tuberculosis* strain (123, 141–143). Mice deficient in PGE2 synthase infected with *M. tuberculosis* show greater susceptibility with high bacterial loads in the lung (132, 142), whereas mice deficient in 5-lipoxygenase (the metabolizer of arachidonic acid into LXA4 and leukotriene B4) are more resistant to *M. tuberculosis* infection with lower bacterial loads in the lung (124, 141). Recent work in zebrafish infected with *M. marinum* revealed that Ita4h (leukotriene A4 hydrolase) mutations that result in increased LXA4 production led to reduced host resistance to mycobacterial infection (111). The discovery that polymorphisms in *Alox5* and *Ita4h* confer susceptibility to human TB supports the relevance of these collective findings to human disease (111, 168). Thus, two important areas for research remain: how the eicosanoid pathways are regulated (α) by other host factors, including cytokines produced during the innate and adaptive immune response to *M. tuberculosis*, and (β) by the genetic variations in the pathogen itself.

**Matrix metalloproteinases in tuberculosis.** The biochemistry of the lung extracellular matrix predicts that matrix metalloproteinases (MMPs) will be among the proteases that contribute to lung matrix destruction in TB (169). MMP-1 is a key collagenase upregulated in patients with TB and associated with increased lung pathology in transgenic mice (170, 171). MMP-9 has been implicated in the pathogenesis of several inflammatory diseases and is highly expressed in TB (172). In humans, MMP-9 activity has been correlated with worse outcomes in TB, suggesting a role in susceptibility to *M. tuberculosis* infection (173; reviewed in 172). In zebrafish, MMP-9 regulates monocyte recruitment to the granuloma (174), indicating that MMPs both modulate the immune response to *M. tuberculosis* and drive pathology (169). Doxycycline suppresses MMP-1 and MMP-9 secretion in vitro and may hold promise as a therapeutic modulator of the MMP-mediated host response to *M. tuberculosis* infection (175).

**Vitamin D and the immune response in tuberculosis.** 25-hydroxyvitamin D [25(OH)D3] is a prohormone whose availability in humans is primarily determined by UV conversion of 7-dehydrocholesterol in the skin. It is transported while bound to vitamin D binding protein (VDBP) and converted renally to its active form [1,25(OH)D3] by 1α-hydroxylase (CYB27B1). Conversion can also occur in granulomatous tissue. Historically, sunlight exposure and vitamin D were used as treatments for TB (121), and clinical trials showed moderate efficacy in some populations, such as those with particular vitamin D receptor polymorphisms, when these treatments were added to standard antitubercular therapy (176, 177). Vitamin D deficiency is associated with the risk of active TB in several populations (178), and polymorphisms in both the vitamin
D receptor (VDR) and VDBP are associated with an increased risk of TB (179).

1,25(OH)\(_2\)D\(_3\) has many regulatory and anti-inflammatory immune effects, including antagonism of \(M.\) \(tuberculosis\)-induced Th1 immunity, which is necessary for protection from disease (121). However, there is a synergy between, and a necessity for, 1,25(OH)\(_2\)D\(_3\) in IFN-\(\gamma\)-mediated restriction of intracellular growth of \(M.\) \(tuberculosis\) in vitro (119, 180). Ligation of TLR2 induces CYP27B1 and thus 1,25(OH)\(_2\)D\(_3\)-mediated induction of the antimicrobial peptide cathelicidin, which restricts the growth of \(M.\) \(tuberculosis\) directly (120) and via the induction of autophagy (181). This combination of anti-inflammatory and bacteriostatic effects of vitamin D therefore continues to be of potential therapeutic interest.

**PATTERN-RECOGNITION RECEPTORS, ADAPTOR PROTEINS, AND IL-1**

Several types of pattern-recognition receptors (PRRs) are involved in host recognition of \(M.\) \(tuberculosis\), including Toll-like receptors (TLRs); C-type lectin receptors (CLRs), including dectin-1, mannose receptor, and DC-SIGN; and Nod-like receptors (NLRs) (reviewed in 125, 182-185; see also 186, 187). Human studies have suggested that genetic variation in genes encoding for PRRs and downstream signaling molecules may affect disease susceptibility, severity, and outcome, but which genes and variants are actually associated with susceptibility to TB is only partially understood (188). Both MyD88 (189-191) and CARD9 (master adaptors of TLR and caspase-recruitment domain family signaling, respectively) (192) are critical for protective immunity to \(M.\) \(tuberculosis\) infection in mouse models.

In MyD88\(^{-/-}\) mice, the loss of resistance to \(M.\) \(tuberculosis\) infection was associated with impaired IL-12, TNF, and Th1 cytokine production and impaired iNOS expression (190) and with reduced TNF, IL-12, and nitric oxide production in \(M.\) \(tuberculosis\)-infected macrophages and DCs (189). Among the TLR family, TLR2, TLR4, and TLR9 play a role in host recognition of \(M.\) \(tuberculosis\); however, the effect of mutations in these TLRs on the in vivo immune response and disease outcome in mouse models of \(M.\) \(tuberculosis\) infection has been minimal and variable (reviewed in 125, 182, 183). IL-1 signaling is required for resistance against \(M.\) \(tuberculosis\) infection (193-195). Indeed, IL-1 signaling is the critical component of the MyD88-dependent innate response to \(M.\) \(tuberculosis\) (125, 196), which is required for resistance to \(M.\) \(tuberculosis\) infection in vivo (197, 198) and is induced via a caspase-1-independent mechanism (198). The critical role of IL-1 in protection against \(M.\) \(tuberculosis\) has recently been reviewed in more depth (124).

The adaptor CARD9 plays a key role in protection against \(M.\) \(tuberculosis\) infection (192) by converging signals from multiple PRRs. Compared with control mice, Card9\(^{-/-}\) mice showed a higher \(M.\) \(tuberculosis\) burden relatively early after aerosol infection, with a higher mycobacterial burden, pyrogranulomatous pneumonia, accelerated granulocyte recruitment, and higher abundances of proinflammatory cytokines and G-CSF in serum and lung (192). Card9\(^{-/-}\) granulocytes failed to produce IL-10 after \(M.\) \(tuberculosis\) infection, suggesting that an absent anti-inflammatory feedback loop may contribute to granulocyte-mediated pathology. NOD2-deficient mice appear to have impaired resistance to \(M.\) \(tuberculosis\) infection through impaired innate and adaptive immunity (186).

Although TLRs may not play an essential role in protection against \(M.\) \(tuberculosis\) infection per se, signals delivered through TLRs by the mycobacteria may modulate the immune response via TLR to the mycobacteria’s advantage, with an effect on innate immune susceptibility, lung pathology, and cytokine expression at the site of infection. Investigators have suggested that the Beijing strains of \(M.\) \(tuberculosis\) are more virulent than other \(M.\) \(tuberculosis\) genotypes, inducing nonprotective immune responses, yet the different Beijing strains themselves elicit highly heterogeneous immune responses (24, 25).
forthcoming report shows certain Beijing strains of *M. tuberculosis* that preferentially activate TLR2 and others that activate TLR4 (J. Carmona & M. Saraiva, manuscript under revision). Recognition of *M. tuberculosis* strains by TLR4 resulted in a distinct cytokine profile in vitro and in vivo, and TLR4 exercised an early protective role in vivo when infecting with TLR4-activating Beijing *M. tuberculosis* strains. Thus, different strains of the *M. tuberculosis* Beijing lineage may use differential signaling through TLRs to their advantage.

**ADAPTIVE IMMUNE RESPONSE IN TUBERCULOSIS**

**A Requirement for T Cells in the Protective Immune Response to *M. tuberculosis* Infection**

Protective immunity and delay of control of bacterial growth during *M. tuberculosis* infection depend on CD4$^+$ T cells because CD4$^+$ T cell–deficient (or MHC class II–deficient) mice are unable to control bacterial growth and thus succumb to disease, and CD4$^+$ T cell lymphopenic HIV patients are highly susceptible to TB (11, 13, 89). CD8$^+$ T cells also contribute to anti-*M. tuberculosis* immunity (13), potentially by secreting IFN-γ to activate macrophages to control infection and/or by secreting products that can directly kill the *M. tuberculosis* bacilli. However, CD8$^+$ T cells clearly cannot compensate for a lack of CD4$^+$ T cells (reviewed in 12, 13). *M. tuberculosis* lipid antigens can also be processed and presented to unconventional T cells such as γδ T cells and NKT cells, but their role in the immune response to *M. tuberculosis* is still unclear (199, 200).

**The Role of DCs in the Immune Response to *M. tuberculosis* Infection**

Because DCs are the prime antigen-presenting cells in the initiation of T cell responses (201), their role in the initiation of the immune response to *M. tuberculosis* is expected. However, how they initiate this response is complex in the context of their interaction with apoptotic macrophages and neutrophils containing *M. tuberculosis* bacilli, which accelerates bacterial acquisition by DCs, earlier trafficking of these DCs to lymph nodes, and faster T cell priming (143, 150). DCs in lymph nodes from patients with TB may themselves contain *M. tuberculosis* (202). Furthermore, depletion of CD11c$^+$ cells in mice before intravenous infection with *M. tuberculosis* delays the development of CD4$^+$ T cell responses and results in impaired control of *M. tuberculosis* (203). More recently, using GFP-expressing *M. tuberculosis*, Wolf and colleagues (116) demonstrated that DCs are indeed infected with *M. tuberculosis* at high frequency in the lungs and lymph nodes after aerosol infection, with a peak in the draining lymph nodes 3 weeks after infection (Figure 4). However, the subsets of DCs that were infected at high frequency in vivo poorly stimulated *M. tuberculosis* antigen–specific CD4$^+$ T cells, despite expression of surface MHC class II and costimulatory molecules (116).

DC responses are also influenced by the *M. tuberculosis* strain itself, and the early events following infection. The level and form of DC activation will determine the magnitude, timing, and class of the resulting immune response to *M. tuberculosis* infection in addition to determining whether control of the infection is achieved or chronic infection and disease progression ensue. Thus, although DCs are clearly fundamental in initiating immune responses to *M. tuberculosis* infection (203), they are regulated at various checkpoints of the host immune response, as described above, and at various stages discussed later throughout this review (and see Figures 4 and 5).

**Antigen-Specific CD4$^+$ T Cell Responses Are Initiated in Local Lymph Nodes After Aerosol *M. tuberculosis* Infection**

It is widely recognized that there is a considerable delay in the onset of detectable
Figure 5

Regulation of the immune response during *M. tuberculosis* infection. Following infection with *M. tuberculosis*, specific regulatory pathways that normally serve to limit host-induced immune pathology may inadvertently promote pathogen persistence. Two such regulators include IL-10 and regulatory T cells. The induction of IL-10 during infection can lead to the inhibition of macrophage effector functions, with reduced bacterial killing and impaired secretion of cytokines/chemokines. IL-10 can also block chemotactic factors that control DC trafficking to the draining lymph nodes. In the lymph nodes, both IL-10 and regulatory T cells can block the differentiation of naive T cells to IFN-γ-producing Th1 cells, predominantly through direct effects on the DC. Furthermore, IL-10 can block T cell chemotactic factors such as CXCL10, which mediates Th1 cell trafficking back to the lungs, in addition to blocking macrophage activation and downstream antimicrobial pathways in response to IFN-γ.

T cell responses in humans and in experimental models of TB when compared with other lung infections (reviewed in 204, 205) (Figure 4). Using adoptive transfer of *M. tuberculosis* antigen–specific TCR-transgenic CD4⁺ T cells, investigators showed that 7–11 days after aerosol infection with *M. tuberculosis*, initial activation of antigen-specific CD4⁺ T cells occurs in the local lung-draining mediastinal lymph node, where their numbers rapidly increase before they traffic to the lung (206–208). Activation of antigen-specific CD4⁺ T cells depends on presentation of *M. tuberculosis* antigens in the lymph node, despite the presence of 100-fold more bacilli in the lungs, and this delay leads to consequent dissemination to the spleen (208). In addition to the reported delay in the T cell response to antigens in the draining lymph node (133, 205–208), even fully differentiated IFN-γ-producing Th1 cells that are transferred prior to *M. tuberculosis* infection (so that large numbers are present in
the lung) do not control bacterial replication until 7 days after infection (206).

Multiple mechanisms may explain the delay in the onset of T cell activation and functional effector T cell responses after primary (133, 205–208) or secondary (209) infection with *M. tuberculosis*. Such mechanisms include *M. tuberculosis* inhibition of apoptosis of macrophages (143) and neutrophils (150), as discussed above, which delays or inhibits the antigen-presenting capacity of DCs; Foxp3+ regulatory CD4+ T cells, which not only inhibit (133, 210, 211) but also delay the arrival of effector T cells in the lung during early TB (133, 204) (Figure 5); and IL-10, which inhibits and delays production of the cytokines IFN-γ and IL-17 by CD4+ T cells in the lung (153, 212) (Figure 5). Many of these mechanisms also operate via effects on DC migration and function.

**CD1-Restricted Responses in *M. tuberculosis* Infection**

Notably, the T cell response to *M. tuberculosis* infection is somewhat unusual in that much of the response is directed at glycolipid antigens presented by the CD1 family of antigen-presenting molecules, as opposed to being directed only at peptide antigens (reviewed elsewhere, e.g., 11) presented by classical MHC class I and II (213). This distinction is due to the large glycolipid component of the *M. tuberculosis* cell wall and plasma membrane and to the fact that the intracellular location of *M. tuberculosis* intersects the loading pathways of the CD1 molecules (213, 214). Thus, mycobacterial lipids may function as adjuvants and/or cognate T cell antigens. In humans, there are three group 1 CD1 molecules (CD1a, CD1b, and CD1c) and two group 2 CD1 molecules (CD1d and CD1e) (213, 214). Multiple examples of mycobacterial lipids presented by CD1 molecules to human T cells have been reported (214–218). Mice do not express group 1 CD1 molecules and contain only CD1d orthologs, which has hampered investigation into their contribution to the in vivo anti- *M. tuberculosis* response. However, both CD1 transgenic mice infected with mycobacteria (219) and naturally infected cattle generate CD1-restricted T cell responses during infection (57). These findings suggest that glycolipid-reactive T cells play a role in an effective response to *M. tuberculosis*, although beyond their ability to proliferate and produce IFN-γ in response to *M. tuberculosis* glycolipids, they remain largely uncharacterized.

**THE CONTRIBUTION OF B CELL RESPONSES TO THE IMMUNE RESPONSE IN TUBERCULOSIS**

B cells and their production of antibodies are vital to protective immune responses and the efficacy of vaccines against a broad range of pathogens, but their role in the immune response to *M. tuberculosis* infection has been elusive (220). Although *M. tuberculosis* is an intracellular pathogen controlled by various intracellular mechanisms activated in macrophages by cytokines, such as IFN-γ (12), B cells likely play a greater role in the host defense against *M. tuberculosis* infection than previously thought (220). In this context, follicle-like B cell aggregates have been observed in the lungs of TB patients (104) and in the granulomas of mice infected with *M. tuberculosis* (220). More recently, investigators have identified activated B cells in the granulomas of nonhuman primates infected with *M. tuberculosis* (221), and B cells and their antibodies likely orchestrate local host defense and/or immunomodulation in the lung of *M. tuberculosis*-infected hosts (220). Studies of *M. tuberculosis* infection in B cell–deficient mice have yielded various results, with reports of diminished immunity, of delayed pathologic progression, and of no apparent effect (reviewed in 220). As with other immune modulators discussed throughout this review, these inconclusive findings may reflect the genetic background of the mouse, animal housing conditions and flora, and the dose and/or strain of *M. tuberculosis* used in the various studies (222, 223). B cells moderate inflammatory progression and enhance containment upon
pulmonary challenge with *M. tuberculosis* (223). Thus, B cells could be modulating immune activation and susceptibility to infection via immune regulation by the induction of cytokines such as IL-10, possibly by engagement of distinct FcyR by antibodies produced by B cells during *M. tuberculosis* infection (220, 223, 224). B cells and the antibodies they produce may differentially affect immune responses toward protection and/or pathogenesis depending on the stage and dose of infection, and this will undoubtedly be affected by the genetic background of the host and the *M. tuberculosis* strain.

**CYTOKINES IN ADAPTIVE IMMUNITY TO M. TUBERCULOSIS**

**IL-12, IFN-γ, and the Th1 Axis Are Required for Protective Antimycobacterial Responses**

Protective immune responses against *M. tuberculosis* are largely mediated by CD4+ T cells, which secrete IFN-γ (reviewed in 12). Antigen-specific CD8+ T cells, natural killer (NK) cells, γδ T cells, and CD1-restricted T cells also produce IFN-γ during *M. tuberculosis* infection, but, as discussed above, they cannot compensate for a lack of CD4+ T cells (11–13). Mice are unable to control a low-dose *M. tuberculosis* infection in the absence of IFN-γ (88, 225). They fail to produce reactive nitrogen and oxygen intermediates, and they develop progressive tissue destruction, which is associated with uncontrolled bacterial replication.

The induction of protective IFN-γ T cell responses against primary *M. tuberculosis* infection is dependent on IL-12 (p40/p35) (11, 12, 87, 124, 226), which is mainly secreted by *M. tuberculosis*-activated DCs (11, 124), in part via TLR-dependent mechanisms (227). Mice lacking IL-12p40 cannot control the growth of the bacterial infection (11, 87, 124). Not only is IL-12 essential for the initial activation of IFN-γ T cell responses to *M. tuberculosis*, but continued IL-12p70 production is also required for the expanded and sustained IFN-γ Th1 responses in the lungs that are required to maintain control of chronic infection (227). Further studies have revealed different contributions of the p40 and p35 subunits of IL-12 to the control of mycobacterial infection (228, 229). Mice lacking the p40 subunit were more susceptible to *M. tuberculosis* infection than were p35-deficient (IL-12−/−) mice and showed increased bacterial growth, increased mortality, and reduced IFN-γ T cell responses compared with the p35−/− mice (230). This finding suggested that IL-12p40 itself may play a protective role. More recently, IL-12p40 was found to be required for DC migration and T cell priming during *M. tuberculosis* infection (226). An alternative explanation for the greater susceptibility of p40−/− than p35−/− mice to *M. tuberculosis* infection is that IL-23p19 contributes to protective immunity. IL-23p19 binds with IL-12p40 to form functional IL-23, and IL-23p19 is expressed early during *M. tuberculosis* infection (231). However, IL-23p19−/− mice effectively controlled *M. tuberculosis* infection, and there was no reduction in IFN-γ-specific T cells or IFN-γ mRNA at the site of infection (232). Conversely, there was a marked reduction in IL-17-producing antigen-specific CD4+ T cells and IL-17 mRNA expression in the lungs. As IL-23p19 is not required to control *M. tuberculosis* infection, it is not clear whether Th17 responses play a significant protective role (232) (discussed further below). In the absence of IL-12p70, IL-23 could compensate for the generation of IFN-γ-producing cells during *M. tuberculosis* infection (232), although this compensatory response was insufficient to control the infection (230, 232). Therefore, although IL-23 can partially compensate for IL-12p70 deficiency to stimulate a Th1 response, this cytokine is not essential to control mycobacterial infection.

**Mutations in the IL-12/IFN-γ Axis Increase Susceptibility to Tuberculosis**

Mutations in the IL-12/IL-23/IFN-γ axis in subjects with Mendelian susceptibility to mycobacterial disease (MSMD) predispose
otherwise healthy subjects to progressive infection with the vaccine strain \textit{M. bovis} BCG and environmental nontuberculous mycobacteria (NTM) (reviewed in 233–235). Whereas complete IFN-γ receptor 1 (IFN-γR1) deficiency has been reported to cause fatal lepromatoid BCG infection and disseminated NTM, partial IFN-γR1 deficiency resulted in clinical TB in a sibling of a child with tuberculoid BCG infection (236). The area of research concerning the IL-12/IL-23/IFN-γ axis in subjects with MSMD predisposing to BCG infection and disseminated NTM has already been extensively reviewed (233–235). Because the present review focuses on disease resulting mainly from \textit{M. tuberculosis} infection, this section describes studies on inborn errors of immunity that result in TB disease specifically [Mendelian susceptibility to tuberculosis (MST)].

As in the case of MSMD, the first evidence that severe primary TB may be caused by single-gene inborn errors of immunity (MST) was provided by a series of publications describing children with IL-12 receptor β1 (IL-12Rβ1) deficiencies that prevented cellular responses to IL-12 and resulted in the patients developing disseminated TB, even though they were apparently resistant to BCG and atypical mycobacterial infection, in contrast to their siblings who had developed these diseases (14, 15, 237–240). At the population level, in at least two countries with high rates of consanguineous marriages (Morocco and Iran), severe TB may result from an autosomal recessive IL-12Rβ1 deficiency in some children (2 out of 50) (238). However, the case of two Spanish siblings with TB and IL-12Rβ1 deficiency, born to nonconsanguineous parents, suggests that there may be more cases in the world (239) other than in countries with high rates of consanguinity. The prevalence of TB in IL-12Rβ1-deficient patients (MST) is lower than that of disease that is due to BCG or nontuberculous mycobacterial infection (MSMD) (14), possibly because patients are exposed less frequently to \textit{M. tuberculosis} than to the BCG vaccines and/or environmental nontubercular mycobacteria.

Other mutations have recently been described that also contribute to MSMD and/or MST. These include previously unknown mutations in \textit{CYBB}—the human gene encoding the gp91phox subunit of the phagocytic NADPH oxidase—which result in an impaired respiratory burst in monocyte-derived macrophages (and presumably tissue macrophages), but not in monocytes or granulocytes, and are essential for immunity against BCG and for resistance to \textit{M. tuberculosis} infection (241). Mutations in IFN regulatory factor 8 (IRF8) that result in DC deficiency underlie disseminated infections caused either by BCG vaccination or by severe combined immunodeficiency (SCID), depending on whether the genetic form of the mutation is autosomal dominant (BCG) or autosomal recessive (SCID) (241). Mice with mutant \textit{Irf8} are susceptible to infection with intracellular pathogens, including \textit{M. tuberculosis} (242). The lack of mycobacterium-induced ISG15 secretion by leukocytes, particularly granulocytes, reduces the production of IFN-γ by lymphocytes, including NK cells, resulting in enhanced susceptibility to mycobacterial disease (243). ISG15 is an IFN-α/β-inducible, ubiquitin-like intracellular protein that, together with various proteins (ISGylation), contributes to antiviral immunity in mice. Interestingly, however, patients with inherited ISG15 deficiency present with mycobacterial, but not viral, diseases, illustrating that human ISGylation is largely redundant for antiviral immunity but plays an essential role as an IFN-γ-inducing secreted molecule for optimal antimycobacterial immunity. The clinical and immunological phenotypes of ISG15-deficient patients resemble those of patients with IL-12p40 or IL-12Rβ1 deficiency, with impaired but not abolished IFN-γ immunity, and with relatively mild MSMD, and \textit{Isg15}-deficient mice are highly susceptible to \textit{M. tuberculosis} infection (243).

Although TB in children and adults generally constitutes two differing epidemiological and clinical forms of disease, an IL-12Rβ1-deficient patient presenting with disseminated TB as an adult (aged 33 years) may be
informative (244). The patient presented with fever, generalized lymphadenopathy, and hepatosplenomegaly. Although he had a history of antituberculous treatment in the previous three years, he did not have any other relevant medical history and had a normal chest X-ray; however, he was smear-positive for acid-fast bacilli and positive by PCR for *M. tuberculosis* complex. A drug susceptibility test revealed resistance to isoniazid and rifampin. The patient died of disseminated TB despite antibiotic therapy. The same group and collaborators describing the case have mapped the first adult TB-linked locus, a major susceptibility locus on chromosome 8 conferring dominant susceptibility to pulmonary TB in adults from Morocco (245).

These experiments of nature in humans provide a wealth of information as to the host genetic factors underlying susceptibility to TB and other mycobacterial diseases, as well as information on the different presentations of the disease. Although susceptibility to *M. tuberculosis* and its clinical expression in adults may often reflect the underlying human genetic background, there are environmental factors and other factors, such as the genetics of the *M. tuberculosis* pathogen itself and the underlying predisposition of the patient through coinfection with other organisms such as HIV, that undoubtedly also determine susceptibility to adult TB.

**Production of IFN-γ During *M. tuberculosis* Infection in Humans**

IFN-γ is the cytokine most invariably detected as protein or mRNA at the sites of human *M. tuberculosis* infection (including in the lung, bronchoalveolar lavage (BAL) fluid, TB pleuritis fluid, and lymph nodes) and in the responses of PBMCs to mycobacterial antigens (reviewed in 246). Although IFN-γ is critical for protection against *M. tuberculosis* infection, IFN-γ levels are highest in the BAL fluid of patients with more severe TB disease (247) and fall in accordance with successful therapy (247). In addition, some studies have shown that, compared with healthy controls, plasma IFN-γ levels are elevated in patients with active TB (248, 249); in one study, however (72), IFN-γ was not detectable in the serum of active TB patients recruited in London. These different findings could reflect the extent of disease, given that, when IFN-γ levels were detectable, they were highest in patients with more advanced TB before drug therapy (248, 249) and were diminished after successful anti-TB chemotherapy (249). That IFN-γ levels correlate with the extent of active TB disease (248, 249) is in keeping with our findings that the blood transcriptional IFN-inducible gene signature correlated with the extent of radiographic lung disease and was diminished upon treatment (72) and also suggests a greater sensitivity and therefore reproducibility of the transcriptome approach.

Despite both the consistent association of increased IFN-γ production by human lung cells in response to *M. tuberculosis* antigens with active TB and the increased levels of IFN-γ in lung fluid and serum from active TB patients, several studies have demonstrated a relative depression of the in vitro IFN-γ recall responses of PBMCs, both to mycobacterial antigens and to mitogens in newly diagnosed active TB patients compared with asymptomatic latent individuals or controls (250–256). This depression in IFN-γ responses by PBMCs was associated with more severe TB disease (257, 258), and the depressed responses were restored to those of asymptomatic exposed latent individuals in some subjects following successful antitubercular chemotherapy (252, 254, 257). One possible explanation is that PBMCs from active TB patients are more susceptible to apoptosis than are those from healthy controls (259), resulting in reduced numbers of IFN-γ-secreting cells. Alternatively, the decrease in the IFN-γ T cell responses of PBMCs during pulmonary TB may suggest that effector T cells are recruited to the lung and sequestered there. This sequestration could explain why the reactivity of PBMCs from TB patients is reduced most profoundly in patients with the most active disease—cases in which a strong Th1 response is observed in the lungs—and that this response is restored in
PBMCs after the infection subsides following therapy.

In contrast, a few reports indicate intact or even heightened IFN-γ production by PBMCs stimulated with *M. tuberculosis* antigens in active TB patients compared with healthy, tuberculin-reactive controls (260–263). These findings may also reflect the differences in TB disease severity in the patients studied, given that the depressed levels of IFN-γ are more pronounced in patients with more severe disease (257, 258). Severity is a function of the duration of the untreated disease, and early in the course of active disease, there may be an increase in IFN-γ responses because of the increased bacterial load. This increase is observed in *M. bovis* infections of calves in which the levels of IFN-γ T cell responses to the antigen ESAT-6 actually rise with increasing pathology scores and bacterial loads (264). Subsequently, with increased TB disease severity, IFN-γ production and the proliferation of PBMCs in response to *M. tuberculosis* antigens may decrease, eventually leading to unresponsiveness, as recognized in several chronic infections, including leprosy (265) and TB (266–269).

**IFN-γ-Mediated Killing of *M. tuberculosis* Infection in Macrophages**

As discussed above, IFN-γ is essential for the control of *M. tuberculosis* infection (11, 12), and although IFN-γ is known to exert its effects through broad transcriptional programs (270) and the induction of cytokines, the effector mechanisms downstream of IFN-γ signaling responsible for the restriction of mycobacterial infection are still being elucidated. Expression of iNOS is required for host control of *M. tuberculosis* infection in mice (271, 272). Although iNOS-expressing macrophages have been identified in the lungs of humans with TB (273), the correlation between human TB and iNOS expression is still unclear (274). Other IFN-γ-inducible molecules that have been implicated in host control of *M. tuberculosis* infection include LRG-47, an IFN-inducible GTP-binding protein (275). However, further work has demonstrated that Irgm1 (Lrg-47) also regulates the survival of mature effector CD4+ T lymphocytes by protecting them from IFN-γ-induced cell death during *M. tuberculosis* infection (276), suggesting complex roles for Irgm1/Lrg-47 in protection against *M. tuberculosis* infection. However, Irgm1/Lrg-47 appears to be vestigial in humans. IFN-γ is also important for endosome maturation (277) and the induction of antimicrobial peptides (119). Work is ongoing to unravel the role of the intracellular pathways downstream of IFN-γ signaling that are involved in resistance to *M. tuberculosis* infection.

**Control of Inflammation and CD4+ T Cells Is Necessary to Inhibit Host Damage During *M. tuberculosis* Infection**

Because the activity of unrestrained TNF and IFN-γ can be detrimental to the host under conditions of infection or microbial colonization, including during *M. tuberculosis* infection (12), various mechanisms are in place to prevent immunopathology, including those mediated by Foxp3+ regulatory T cells (133, 204, 210, 211) and IL-10 (11, 124, 153, 212). The latter is produced by various cellular sources during *M. tuberculosis* infection.

The balance between these regulators and TNF/IFN-γ may determine if the immune system can eradicate *M. tuberculosis* with minimum pathology.

An excessive inflammatory or immune response to *M. tuberculosis* infection may also result in a lack of mycobacterial control. PD-1 is a cell surface receptor expressed on activated T and B cells upon antigen receptor engagement, which upon binding to its ligands (PD-L1 and PD-L2) delivers a negative signal for proliferation and cytokine production and is recognized as a major regulator of pathogen-specific responses (278). PD-1 is expressed by T cells from TB patients, and PD-L1 expression was induced on T cells stimulated with sonicated H37Rv *M. tuberculosis* (279). In addition,
PD-L1, which interacts with PD-1, is overly abundant in the whole blood of active TB patients compared with healthy controls and latent patients, and expression by neutrophils is largely responsible for this signature (280). Antibodies blocking PD-1/PD-L1/PD-L2 were able to enhance antigen-specific IFN-\(\gamma\) responses in cells from patients with TB in vitro (279), suggesting that PD-1 may contribute to chronic infection, as had already been shown for viral infections (278). However, deletion of PD-1 resulted in very high numbers of \(M.\) \(tuberculosis\)-specific CD4\(^+\) T cells in \(M.\) \(tuberculosis\) aerosol–infected mice (281, 282) but perhaps surprisingly led to increased susceptibility to infection, with CD4\(^+\) T cells themselves driving the increased bacterial loads and pathology seen in the PD-1\(^{-/-}\) mice (281). Thus, although CD4\(^+\) T cells are clearly required for control of \(M.\) \(tuberculosis\) infection, the uncontrolled bacterial growth and cytokine production seen in the absence of PD-1 signaling can also lead to disease susceptibility, demonstrating the importance of a finely regulated immune response to control disease.

A Potential Complex Role for IL-17 and IL-23 During Mycobacterial Infection and Vaccination

Early studies showed that IFN-\(\gamma\)-deficient mice infected with mycobacteria exhibit enhanced accumulation of activated effector T cells and neutrophils within granulomatous lesions (283). These cells did not control bacterial growth and compromised the integrity of the infected tissue, and IFN-\(\gamma\)-deficient mice showed increased numbers of IL-17-producing T cells following \(M.\) \(bovis\) BCG infection (283). These data demonstrated, first, that both IFN-\(\gamma\)- and IL-17-producing T cells are induced during mycobacterial infection and, second, that IFN-\(\gamma\) serves to limit the IL-17-producing T cell population, suggesting that this counterregulation pathway may be an important factor in limiting mycobacterially associated immune-mediated pathology. In keeping with these findings, during \(M.\) \(tuberculosis\) infection, IFN-\(\gamma\) was shown to inhibit CD4\(^+\) T cell production of IL-17, impairing neutrophil survival and the accumulation of pathogenic neutrophils in the infected lung, contributing to decreased lung inflammation and improved disease outcome (154). During mycobacterial infections, IL-17 can also be produced by \(\gamma\delta\) T cells and a non-CD4\(^+\) CD8\(^+\) population (284). IL-17 may play a role in granuloma formation and Th1 enhancement following BCG infection (285) and in granuloma formation during intratracheal infection with \(M.\) \(tuberculosis\) (286). However, IL-23, which is essential for the IL-17 response during TB, is dispensable for protection and antigen-specific IFN-\(\gamma\) responses if IL-12p70 is available (232).

Following vaccination with a mycobacterial peptide, IL-17 is required for the accelerated recruitment of IFN-\(\gamma\)-producing cells to the lung, as a result of increased concentrations of the chemokines CXCL9, 10, and 11, which recruit cells to sites of inflammation (287). Furthermore, Gopal et al. (288) have recently suggested that IL-23-dependent IL-17 is required for the induction of a protective Th1 response to challenge with \(M.\) \(tuberculosis\) following BCG vaccination. If mice are subjected to repeated BCG vaccination after \(M.\) \(tuberculosis\) infection, however, there is increased IL-17, TNF, IL-6, and CXCL2 expression, an influx of granulocytes/neutrophils, and lung tissue damage, with IL-17 and IL-23 mediating immune pathology (152). IL-10-deficient mice infected with \(M.\) \(tuberculosis\) showed an accelerated and enhanced response that was independent of IL-17 (153). Recent data provide strong support for a mycobactericidal effector function of CD4\(^+\) T cells that is independent of the production of IFN-\(\gamma\) or TNF. Furthermore, these data show that the central role of these cytokines is to prime differentiation to the Th1 phenotype, after which other effector functions kill \(M.\) \(tuberculosis\) (289). Notably, transfers of \(M.\) \(tuberculosis\) antigen–dependent Th17 cells only partially inhibit bacterial growth, and Th2 cells had no effect (289), suggesting that
other CD4+ T cell–mediated effector pathways control *M. tuberculosis* infection.

**THE DOUBLE-EDGED SWORD OF SUPPRESSIVE CYTOKINES/FACTORS IN TUBERCULOSIS**

**Interleukin-10 Contributes to Chronic *M. tuberculosis* Infection**

IL-10 is an immunosuppressive cytokine essential for dampening the immune response and limiting host immune pathology to numerous intracellular pathogens and gut flora, but, if overproduced, IL-10 can contribute to chronic infection (290). IL-10 is made by many immune cells including macrophages, neutrophils, DCs, B cells, and T cells (290). A major mechanism whereby IL-10 achieves its effects is by inhibiting the antigen–presenting cell function of macrophages and DCs and the production of cytokines such as IL-12, thus inhibiting the development of Th1 responses (291–293). In addition, IL-10 can inhibit the killing of intracellular pathogens by macrophages, induction of nitric oxide, and production of TNF (293, 294). On the basis of these functions, we can predict a role for IL-10 in the regulation of the immune response to *M. tuberculosis* and disease outcome (212).

Support for a role for IL-10 in the immune response in TB first came from its detection in samples obtained from active TB patients. IL-10 was elevated in the lungs (250, 295, 296), BAL fluid (297), sputum (296), and serum (249) of active TB patients, although its detection in serum was more variable, seen largely in advanced disease. Furthermore, neutralization of endogenous IL-10 in PBMCs obtained from TST+ healthy individuals (298) or active TB patients (299, 300) resulted in increased T cell proliferation and IFN-γ production. PPD-specific CD4+ T cell clones isolated from the BAL fluid of active TB patients produced both IFN-γ and IL-10 upon stimulation (301). Variations in allele 2 of the human gene *SLC11A1* (Nramp1) are associated with increased susceptibility to TB via enhanced production of IL-10 by monocytes (302). However, although polymorphisms in the human IL-10 gene have been suggested as risk factors for TB, conclusive data are lacking (303, 304).

Studies in vivo investigating a role for IL-10 in the control of the immune response to *M. tuberculosis* infection in mice and effects on disease outcome were initially unclear (305, 306). However, IL-10 is clearly induced in vivo in the lungs of both resistant (C57BL/6 and BALB/c) and susceptible (CBA/J) mice infected with *M. tuberculosis* (153, 305, 307, 308). Initial studies reported no difference in bacterial loads in the lungs of *Il10−/−* mice on the TB-resistant C57BL/6 background following *M. tuberculosis* infection (305, 306, 309), whereas others reported transient enhanced levels of IFN-γ and decreased *M. tuberculosis* bacterial loads in the lungs and spleens of *Il10−/−* mice (310). We have confirmed the findings of decreased bacterial loads in lungs and spleens of *M. tuberculosis*–infected *Il10−/−* C57BL/6 mice compared with control mice (153, 212); we have also confirmed them in *Il10−/−* mice on the resistant BALB/c background (153). These distinct findings may be due to differences in the ages and strains of mice used, the initial dose of infecting bacteria, or the microbial flora/status of mice in different laboratories.

Abrogation of IL-10 signaling in *M. tuberculosis*–infected *Il10−/−* BALB/c mice resulted in enhanced levels and earlier production of cytokines associated with protection (IFN-γ, GM-CSF, G-CSF, TNF, IL-17A) and with increased T cell recruitment (CXCL10, IL-17A) (153). The enhanced protection in *Il10−/−* mice infected with *M. tuberculosis* also correlated with early and increased responses of CD4+ T cells in the draining lymph node as well as with an accelerated and elevated production of the chemokine CXCL10 and increased influx/differentiation of Th1 cells into the lung (153) (Figure 5).

IL-10 plausibly functions at many levels to limit the response to *M. tuberculosis* infection (Figure 5), first by inhibiting macrophage and DC function, and then by inhibiting the cytokines/chemokines required for the...
migration of infected myeloid cells to the lymph node and for the migration of Th1 cells from the lymph node to the lung. For example, transferred BCG-infected DCs from \( \text{Il}10^{-/-} \) mice show increased trafficking to the draining lymph nodes in response to mycobacterial antigens, although effects on bacterial load were not assessed (311). \( M. \text{tuberculosis} \) infection of human macrophages induced the production of IL-10 (312, 313) and resulted in the blockade of phagosome maturation via a STAT3-dependent mechanism, resulting in \( M. \text{tuberculosis} \) survival and outgrowth (312). Thus, whereas IL-12p40 promotes DC migration during mycobacterial infection (226), IL-10 may limit it (311) to facilitate \( M. \text{tuberculosis} \) survival.

Despite these proinflammatory responses in \( \text{Il}10^{-/-} \) mice, only limited evidence supports a role for IL-10 in blocking host-mediated immunopathology during chronic \( M. \text{tuberculosis} \) infection (309). Our own work shows that abrogation of IL-10 signaling during \( M. \text{tuberculosis} \) infection of mice does not result in overt immunopathology but allows better control of the pathogen, leading to decreased bacterial loads in the lung and spleen (J.M. Pitt, P.S. Redford, and A. O’Garra, unpublished data).

\( M. \text{tuberculosis} \) infection of genetically resistant C57BL/6 and BALB/c mice results in detectable levels of IL-10 in the lungs within the first 3–4 weeks post-infection (308). Genetically susceptible CBA/J mice have a greater propensity to produce increased levels of IL-10 with decreased levels of IL-12p40 and IFN-\( \gamma \) in the lungs at earlier stages post-infection, and these higher IL-10 concentrations contribute to the enhanced susceptibility of these mice to \( M. \text{tuberculosis} \) infection and to their exacerbated bacterial burdens (308). Blockade of IL-10R signaling during the entire infection (153) or during the chronic phase of disease (307) by administration of anti-IL-10R neutralizing antibodies reduced bacterial burdens and enhanced T cell recruitment, T cell–derived IFN-\( \gamma \), and ultimately host survival.

Bone marrow–derived neutrophils infected in vitro (192) or neutrophils isolated from the lungs of mice challenged with \( M. \text{tuberculosis} \) (151) produce significant levels of IL-10, as do mouse (F. McNab, J. Ewbank, and A. O’Garra, unpublished observations) and human (312, 313) macrophages infected with \( M. \text{tuberculosis} \). Activation through CARD9 (192) and cotriggering of TLR in addition to C-type lectin-Syk-dependent pathways (151) are implicated in the production of IL-10 by neutrophils. We have found that \( M. \text{tuberculosis} \)-induced IL-10 in macrophages is dependent on endogenous levels of type I IFN and that the addition of IFN-\( \beta \) also greatly enhances the production of IL-10 in \( M. \text{tuberculosis} \)-infected wild-type macrophages (F. McNab, J. Ewbank, A. O’Garra, unpublished observations). Activation of TLR4 (but not of TLR2) in macrophages infected with \( M. \text{tuberculosis} \) initiates a program of post-transcriptional regulation of IL-10, leading to an increased stability of IL-10 mRNA with increased IL-10 secretion, and this regulation depends on prolonged activation of the MAPK p38 via the adaptor protein TRIF, activated only downstream of TLR4 and not TLR2 (M. Teixeira-Coelho, J. Guedes, P. Ferreirinha, A. Howes, J. Ewbank, J. Pedrosa, F. Rodrigues, A. O’Garra, A.G. Castro, M. Saraiva, manuscript submitted). Therefore, TLR4-activating \( M. \text{tuberculosis} \) strains will induce higher levels of IL-10 production by macrophages (M. Teixeira-Coelho, J. Guedes, P. Ferreirinha, A. Howes, J. Ewbank, J. Pedrosa, F. Rodrigues, A. O’Garra, A.G. Castro, M. Saraiva, manuscript submitted).

It is unclear which cellular source of IL-10 is responsible for regulation of the immune response to \( M. \text{tuberculosis} \) infection in vivo. Transgenic C57BL/6 mice that overexpress IL-10 in the T cell compartment (under control of the IL-2 promoter) showed enhanced susceptibility to \( M. \text{tuberculosis} \) infection (308). However, \( M. \text{tuberculosis} \) infection of IL-10 transgenic mice where the macrophage compartment specifically overexpresses IL-10 under control of the CD68 promoter also led to increased lung bacterial loads and reduced survival associated with reduced macrophage...
function (314). We have recently tracked the sources of IL-10 in the lungs during *M. tuberculosis* infection using 10BiT reporter mice (315). We detected IL-10 production from monocytes and, to a lesser extent, neutrophils, early after *M. tuberculosis* infection, whereas IL-10 was mostly detectable from CD4^+^ T cells at later stages (P.S. Redford and A. O’Garra, unpublished data).

The induction of IL-10 during *M. tuberculosis* infection may not just be a response initiated by the host in order to limit host-mediated pathologies; it may also benefit the pathogen because different *M. tuberculosis* strains may induce enhanced levels of IL-10 as a mechanism of immune evasion. For example, infection of mice with the *M. tuberculosis* strain HN878 (316) or infection of human monocyte–derived macrophages with the *M. tuberculosis* strain CH (317) produces higher levels of IL-10 when compared with the *M. tuberculosis* H37Rv strain.

BCG vaccination of *Il10^−/−* mice enhances bacterial control after *M. tuberculosis* challenge compared with control mice (288), although whether IL-10 has a regulatory role specifically at the level of initial vaccination is unclear. More recently, we have shown that treatment of *M. tuberculosis*–resistant C57BL/6 and *M. tuberculosis*–susceptible CBA/J mice with an anti-IL-10R monoclonal antibody only during BCG vaccination greatly enhances protection from subsequent *M. tuberculosis* challenge and is associated with enhanced and sustained Th1 and Th17 responses and with the production of IFN-γ and IL-17 by innate-like lymphoid cells (318).

### Type I IFNs and Tuberculosis

The type I IFN family of cytokines are perhaps best known for the induction of antiviral immunity, although they have pleiotropic effects on the broader immune response (323). There has been relatively limited information on the role of type I IFN during human TB. However, we reported that patients with active TB have a prominent type I IFN–inducible gene signature in their blood that correlated with the extent of radiographic disease and diminished upon successful treatment (72) (discussed above), which has added to the literature from experimental *M. tuberculosis* infection that suggests a detrimental role for type I IFN during TB.

In vivo experimental evidence for type I IFN exacerbation of TB has been largely driven by two lines of inquiry: first, *M. tuberculosis* infection of mice lacking the receptor common to all type I IFNs (here denoted as Ifnar1^−/−^ mice) and, second, protocols that lead to heightened levels of type I IFN during infection. Despite some variability between studies, *M. tuberculosis* infection of *Ifnar1^−/−^* mice overall suggests a detrimental role for type I IFN during TB (99, 316, 324, 325). *Ifnar1^−/−^* mice have reduced bacterial load (316, 325, 326) or increased survival (99, 316) after *M. tuberculosis* infection compared with control mice. In addition, mice treated with an anti-IFN-αβ antibody had increased survival post-*M. tuberculosis* infection (99). One study (324) has observed increased bacterial load in *Ifnar1^−/−^* mice infected with *M. tuberculosis*.

### Th2 Cytokines and Tuberculosis

Because Th1 responses are protective against *M. tuberculosis* infection and Th2 responses cross-regulate and inhibit Th1 responses, we should not be surprised by reports that chronic worm infection of mice reduces immunogenicity (319) or by reports of reduced Th1 responses in active (320) and latent (321) TB patients coinfected with helminths. However, although the interaction of helminths with mycobacteria has been reported, the mechanisms underpinning how helminths reduce protective immune responses to *M. tuberculosis* infection are unclear. In this context, a study recently showed that mice infected with the intestinal helminth *Nippostrongylus brasiliensis* placed increased *M. tuberculosis* burdens in the lungs upon coinfection and did so in part by mediating the alternative activation of macrophages via the IL-4R signaling pathway, rather than by decreased Th1 responses (322).
versus controls, but the reason for the disparity with other studies remains unclear.

Overexpression of type I IFN during experimental *M. tuberculosis* infection has provided powerful evidence that uncontrolled type I IFN production is detrimental to the host. The first evidence for this came from studies of infection with hypervirulent strains of *M. tuberculosis* (24, 99, 316), in which induction of increased levels of type I IFN correlated with increased virulence and depressed proinflammatory immune responses. Expanding this work, a recent study shows that enhanced type I IFN induction by hypervirulent *M. tuberculosis* isolates is dependent on differential TLR activation (J. Carmona & M. Saraiva, manuscript under review). In other studies, in which high levels of type I IFN were present, such as with direct instillation of IFN-α/β into the lung of *M. tuberculosis*-infected mice, increased disease resulted (24). Enhanced induction of type I IFN during *M. tuberculosis* infection through administration of a derivative of the TLR3 ligand polyinosinic-polycytidylic acid also led to infection that was more severe, with increased bacterial burden and lung pathology (326). In agreement with these findings, a recent study has shown that deletion of TPL2 (tumor progression locus 2), which acts as a negative regulator of type I IFN production downstream of TLR (327), resulted in exacerbated disease that was dependent on the type I IFN receptor (F. McNab, J. Ewbank, and A. O’Garra, manuscript submitted) (Figure 6).

Type I IFN–mediated changes in cellular populations also appear to be important, with the generation and trafficking to the lung of *M. tuberculosis*-permissive innate cells that contribute to exacerbated disease (326, 331). It is possible that other environmental insults such as acute viral infections or adjuvants that result in increased levels of type I IFN may also exacerbate *M. tuberculosis* infection via a type I IFN–dependent mechanism, expanding the myriad of potential environmental factors, in addition to genetic changes in both host and pathogen, that can result in the development of active TB (Figure 6).

**DIFFERENT M. TUBERCULOSIS STRAINS INDUCE DIFFERENT HOST RESPONSES**

Data from recent studies are emerging that suggest that particular combinations of host (14, 15, 20, 21) and *M. tuberculosis* genotypes (22–26, 99, 332) may be associated with an increased risk of developing active TB and with the level of disease severity (24, 25, 98, 317, 332, 333). Because they have a limited ability to exchange genetic material, *M. tuberculosis* bacilli have evolved primarily by deletion and duplication events, and the consequence is a strongly clonal pattern of evolution with the emergence of separate clonal lineages of strains (23). Recent findings that point to
Type I interferon (IFN) inhibits the macrophage response to *M. tuberculosis*. Production of high levels of type I IFN by macrophages can be induced upon *M. tuberculosis* infection, particularly when the infection is caused by certain hypervirulent strains of *M. tuberculosis*. In addition, separate viral infection of the host may lead to type I IFN production by other cells, such as epithelial cells and plasmacytoid dendritic cells (pDCs). This in turn may lead to the induction of high levels of type I IFN in the microenvironment, which can inhibit macrophage functions such as the production of host-protective cytokines, including IL-12, IL-1 and TNF-α, by signaling through the IFN-α/β receptor (IFNα/βR). Type I IFN also induces macrophage production of IL-10, which similarly inhibits macrophage production of IL-12 and TNF-α. Importantly, type I IFN is also capable of blocking macrophage activation by Th1 cells by impairing responsiveness to IFN-γ.  

Host-pathogen coevolution in TB, which distinguish the *M. tuberculosis* strains into six phylogenetic lineages, also show a subdivision into evolutionarily ancient or modern lineages (22, 23). Such divergence takes the form of SNPs as well as deletion, duplication, and insertion events (334) and could lead to differential pathogenic characteristics being acquired by the different lineages (reviewed in 25). An understanding of the implications of strain diversity has resulted from studies on *M. tuberculosis* strains that have caused TB outbreaks. One such lineage, the W-Beijing, may be emerging worldwide and has distinct phenotypic and genotypic characteristics (24). A member of this lineage, HN878, caused a TB outbreak in the United States in the mid-1990s (335) and was subsequently shown to induce rapid death in mice, with increased mycobacterial dissemination, a reduced Th1 response,
and increased type I IFN production (24). This corresponds with the more recent findings that humans with active TB have a blood signature strongly represented by type I IFN–inducible genes and correlated with the severity of disease (72). Rabbits infected with the HN878 Beijing strain also showed greater disease severity and increased mycobacterial loads in a model of tuberculous meningitis (335).

The increased virulence of this Beijing strain was attributed to a phenolic glycolipid that inhibits the production of proinflammatory cytokines (332), although this glycolipid may not be the only molecule contributing to the virulence of the HN878 Beijing strain (336). Investigators recently demonstrated that aerosol infection of mice with HN878 induced an increased early Th1 response, which was subsequently suppressed, potentially via the induction of IL-10 (316). This may be explained in part by the induction of type I IFN, which we (F. McNab, J. Ewbank, A. O’Garra, unpublished observations) and others (330) have shown can induce IL-10, as already discussed in this review. Infection of macrophages from B6D2F1 mice with the two clinical M. tuberculosis isolates, CDC1551 and HN878 [previously shown to differ in virulence and immunogenicity in mouse and rabbit models of TB (24, 335) but to grow with similar rates], induced transcription of distinct sets of genes (337). CDC1551 induced an increased early global transcriptome consisting of immune response genes and nitric oxide synthase in macrophages by 6 h compared with HN878, which, in contrast, induced a set of host genes involved in lipid metabolism, including cholesterol metabolism and prostaglandin synthesis at 24 h (337). A recent study comparing M. tuberculosis strains from six phylogenetic lineages—also subdivided as evolutionarily ancient or modern lineages—determined that the evolutionarily modern lineages tended toward a significantly lower early inflammatory response during infection of human macrophages, as compared with ancient lineages (333). This overall lower inflammatory response may be a consequence of their access to rapidly increasing numbers of susceptible hosts, which may lead to selection for more rapid progression to active TB (333).

Further transcriptional profiling of mouse macrophages infected with representative high and low inflammation-inducing M. tuberculosis isolates shows that the latter induced a set of genes involved in cholesterol biosynthesis (D. Portevin, D. Young, unpublished data), as described for the Beijing strain (337). As discussed above, strains of the Beijing family are heterogeneous, preferentially activating either TLR2 or TLR4, resulting in differential production of proinflammatory cytokines and type I IFN, showing an early protective role for TLR4 (J. Carmona & M. Saraiva, manuscript under review).

Another strain of M. tuberculosis, CH, caused a large outbreak of TB in Leicester, UK, in 2001 (317). Although less resistant to oxidative and acid stress compared with control strains, CH was unimpaired in its growth in human macrophages. Infection of human macrophages with M. tuberculosis CH resulted in reduced IL-12p40 and increased IL-10, attributable to a particular deletion (25, 317). Determining whether this effect on cytokine production by M. tuberculosis CH is unique or widely conserved in other strains of this East African–Indian lineage will be important, however.

**HIV/TUBERCULOSIS PATHOGENESIS**

Soon after the discovery of HIV-1, investigators realized that TB is the most common opportunistic infection worldwide in HIV-1–infected persons. HIV-1 coinfection predisposes one both to infection by M. tuberculosis and to reactivation of TB, and it modifies the natural history and clinical presentation of TB (338). An increase in extrapulmonary disease is well recognized, but early TB disease characterized by very few or no symptoms is also common (339). Immunodiagnostic methods to ascertain TB sensitization in HIV-1–infected persons are compromised in sensitivity, particularly the TST (340, 341). Antiretroviral therapy
(ART) for HIV-1 infection improves immune resistance to TB but, in patients harboring the M. tuberculosis bacilli or those with active TB, ART not infrequently contributes to pathological immunity known as the immune reconstitution inflammatory syndrome (TB-IRIS) (342).

The most obvious immune defect caused by HIV-1 is a progressive reduction in CD4+ T cell numbers that correlates with both increasing risk of TB and the likelihood of extrapulmonary dissemination. However, and unlike most opportunistic conditions in HIV-1-infected persons, the risk of TB is increased from the time of HIV-1 acquisition, before CD4+ T cell deficiency is profound (343, 344). This raises the question of whether there are additional qualitative defects in CD4+ T cell function. The preferential loss of peripheral M. tuberculosis–specific CD4+ T cells early in HIV-1 infection is attributed to the increased susceptibility of IL-2-producing cells to productive HIV-1 infection (90, 91). This hypothesis is consistent with the report that polyfunctional (i.e., IL-2+, TNF-, and IFN-γ-secreting) CD4+ T cell numbers are greatly decreased in the lungs of highly TB-exposed HIV-1-infected persons when compared with similar HIV-1-uninfected counterparts (345). However, at the site of established TB disease (and of HIV-1 replication), the phenotype of M. tuberculosis–specific CD4+ T cells is skewed toward polyfunctional, potentially because terminally differentiated cells at disease sites express the HIV-1 coreceptor CCR5 and are thus susceptible to lytic HIV-1 infection (346). Mechanisms of HIV-1–associated susceptibility to TB have hitherto been difficult to model in animals. The advent of a low-dose aerosol infection of SIV-infected macaques has revealed HIV-1–mediated disruption of granuloma formation similar to that of human disease (347, 348).

The susceptibility of HIV-1-infected patients to reinfection by M. tuberculosis before CD4+ T cell depletion raises the possibility that HIV-1 induces an innate immune defect. Findings on the effect of HIV-1 infection on the ability of macrophages to restrict the growth of M. tuberculosis appear dependent on experimental circumstances, with some reporting increased replication (349) while others show little effect (345, 350). A more recent report has proposed that M. tuberculosis increases HIV trans-infection and induces viral sequestration within surface-accessible compartments in DCs (351). In addition, vitamin D reportedly inhibits HIV-1 and M. tuberculosis infection in macrophages through the induction of autophagy (352).

There is consensus that M. tuberculosis increases the intracellular replication of HIV-1 via cytokine- and chemokine-mediated mechanisms (353, 354) and via the loss of an inhibitory transcription factor, CCAAT/enhancer-binding protein beta (C/EBPβ), activation of nuclear factor (NF)-κB, and positive transcription elongation factor (P-TEFβ) (355–357).

ART reduces susceptibility to TB in HIV-1-infected persons via viral suppression, thus allowing partial immune restoration. Early reports documented expansion of terminally differentiated tuberculin PPD-specific CD4+ T cells by flow cytometric analysis in TST-positive patients during ART (358). Subsequent work has shown that ART is associated with expansion of both terminally differentiated and effector memory M. tuberculosis antigen–specific CD4+ T cells (359, 360). Conversely, the frequent occurrence of TB-IRIS has brought into focus again the importance of pathological immunity in TB and how poorly it is understood in humans. Clues come from the risk factors for TB-IRIS: disseminated TB, a low CD4+ count prior to ART, and a shorter interval from TB treatment to ART (361). These features suggest that TB-IRIS is driven by increased recognition of abundant M. tuberculosis antigens. TB-IRIS is associated with the conversion of a negative TST response to a strongly positive one after ART (362).

Large PPD-specific Th1 expansions can be demonstrated in vitro during TB-IRIS, which investigators initially ascribed as the cause of TB-IRIS (363). However, such expansions also occur frequently in patients who develop no symptoms and do not relate well to symptom resolution, bringing into question whether this striking phenomenon is truly causal (364, 365).
Another hypothesis is that TB-IRIS is associated with defective restoration of regulatory T cell function. However, there appears to be no deficit in the numbers of CD4+ Foxp3+ T cells in TB-IRIS patients when compared with similar patients who do not develop the syndrome (365, 366). Recent interest has therefore turned to the interaction between reconstituting innate and acquired responses in the induction of this syndrome (367). There is evidence of myeloid activation, in particular a signature driven by IL-6 and TNF (281, 368, 369). Other studies have implicated the cytolytic function of NK cells as well as killer immunoglobulin receptor (KIR)–negative γδ T cells in the induction of TB-IRIS (370, 371). Continued study in this field appears to represent an opportunity to learn about tissue-damaging immune responses that would be an undesirable consequence of novel vaccination strategies.

**DIFFICULTIES AND ADVANTAGES IN STUDYING THE IMMUNE RESPONSE IN TUBERCULOSIS IN BOTH EXPERIMENTAL MODELS AND HUMAN DISEASE**

So where are we heading with respect to improving our understanding of the immune response in TB? Much work remains regarding (a) the genetics of the host and the pathogen itself; (b) host-pathogen interactions that result in control of infection; and (c) conversely, the factors that result in the 5–10% of infected individuals progressing to active TB. Progress in these areas is needed before we can make advances in diagnosis, prognosis, and therapy. Can we determine the host factors that allow the approximately 2 billion latently infected individuals to remain healthy after infection with *M. tuberculosis*, and can we use this information to determine correlates of protection useful to vaccination? Should such correlates be determined by standard and advanced immunological techniques, transcriptomics, genetic approaches, or a combination of approaches? As discussed in this review, changes in the immune response will be determined by genetic differences in the pathogen and the host, which may be addressed using the new high-throughput sequencing techniques that have evolved in the past few years. However, to make sense of effects resulting from these genetic differences, we must have a clear definition of the clinical disease phenotype, along with a global picture of the accompanying changes in the immune response at different stages and states of disease, with the acknowledgment that active and latent TB present as heterogeneous conditions. Knowledge from all experimental models is essential, ranging from zebrafish, mouse, and guinea pig to rabbit and nonhuman primate. We must, of course, recognize the advantages and the limitations of each model, make appropriate comparisons across the species, and modify each accordingly to more closely resemble the human disease (Figure 7).

Research into the immune response in TB should therefore be an iterative process with improved understanding of human TB leading to the improvement of experimental animal models. Such experimental models are essential to study the immune response in the whole organism and not just in the blood and those limited tissues that one can obtain from humans with TB. Studies of the immune response in the blood in experimental models are needed, as are comparisons with both events in infected tissue, such as the lung in pulmonary TB, and events that control or allow dissemination of mycobacteria for the benefit or detriment of the host. The mouse has advantages over other experimental models because of the wealth of genetics and reagents available, but using this model requires increasing studies in TB-susceptible genetic mouse strains and infection with *M. tuberculosis* strains more closely related to clinical isolates. Comparison of disease induced by infection with clinical isolates of *M. tuberculosis* may help to elucidate pathways leading to pathogenesis. And we must consider that the culture conditions used to expand the mycobacteria for studies in the laboratory may vary their phenotype and/or genotype away from the microorganism that is coughed.
Integrating systems biology approaches with mechanistic studies and experimental models to advance our understanding of the immunopathogenesis of tuberculosis (TB). Despite the extensive work detailed in this review, our understanding of TB remains incomplete. Current animal models fail to fully recapitulate all features of the human disease, and the anatomical location of the infection in humans makes human studies difficult. Mechanistic studies have tended to focus on the same few candidate cells and cytokines and have therefore struggled to provide effective new vaccine candidates or biomarkers. Using comprehensive profiling tools, such as microarrays, sequencing, and proteomics, to gain an unbiased survey of the human response to \textit{M. tuberculosis} in vivo and making comparisons with lung pathology will allow animal models to be modified and improved to more accurately reflect the human disease. Furthermore, studying identical compartments (such as whole blood or PBMCs) in animals and humans will enhance our ability to make direct comparisons between model systems and human disease. These broad surveys can then direct mechanistic studies toward novel areas for the development of therapeutic agents, diagnostic biomarkers, and vaccines.

up from one individual to another during transmission of \textit{M. tuberculosis}. Furthermore, how the infectious bacterial load may affect the subsequent immune response is still unclear. Although the standardization of growth in different labs may help reduce variation, comparison of different studies could still be confounded by the different statuses of various animal houses, microbial flora \cite{372}, body temperatures of the mice \cite{373}, and the aerosol modes and doses used in TB infection models. However, provided that one attempts to relate findings about the immune responses in experimental models back to the immune response occurring during the different stages of human TB, we will be able to use the information to increase our knowledge of this complex disease and to move toward improved diagnosis, prognosis, drug treatment, and vaccination.
DISCLOSURE STATEMENT

A.O.G., M.P.R.B., R.J.W., and C.I.B. are coinventors on patents relating to use of transcriptional signatures for potential diagnosis and treatment monitoring in TB and other lung diseases. The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED


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52. Reichman LB. 1979. Tuberculin skin testing. The state of the art. *Chest* 76:764–70
87. Cooper AM, Magram J, Ferrante J, Orme IM. 1997. Interleukin 12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with Mycobacterium tuberculosis and relationship to major histocompatibility complex haplotype and Nramp1 genotype. Immunology 93:270–74

115. 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* 137:122–28


228. Cooper AM, Kipnis A, Turner J, Magramp, J, Ferrante J, Orme IM. 2002. Mice lacking bioactive IL-12 can generate protective, antigen-specific cellular responses to mycobacterial infection only if the IL-12 p40 subunit is present. *J. Immunol.* 166:1322–27
232. Khader SA, Pearl JE, Sakamoto K, Gilmartin L, Bell GK, et al. 2005. IL-23 compensates for the absence of IL-12p70 and is essential for the IL-17 response during tuberculosis but is dispensable for protection and antigen-specific IFN-γ responses if IL-12p70 is available. J. Immunol. 175:788–95


284. Lockhart E, Green AM, Flynn JL. 2006. IL-17 production is dominated by γδ T cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. *J. Immunol.* 177:4662–69


*J. Infect. Dis.* 195:1303–10


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