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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.

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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**).

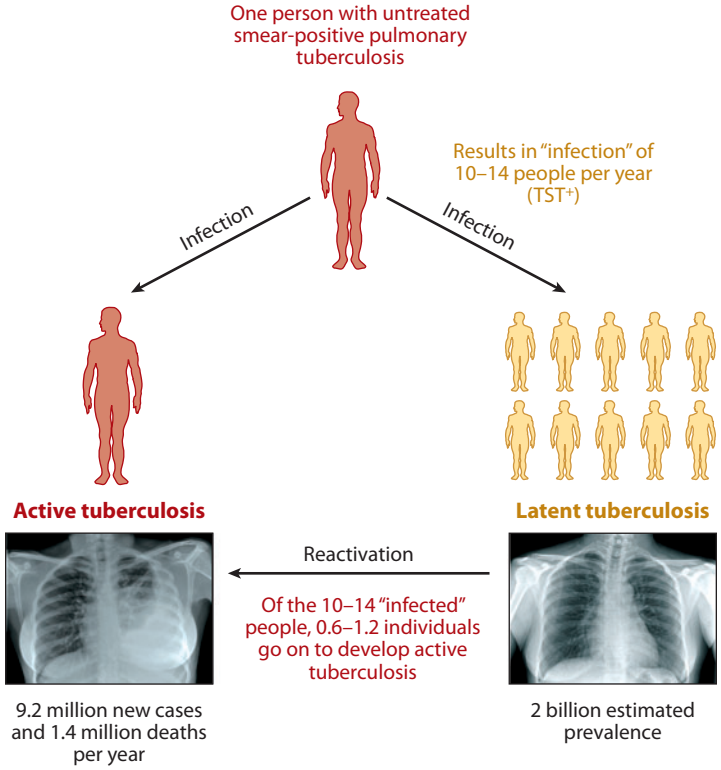

Figure 1

The traditional epidemiology of *M. tuberculosis* infection—active and latent TB. TB disease results from infection with the pathogen *M. tuberculosis*, which is spread by respiratory transmission. The active form of the disease is characterized by 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 (diagnosed by detection of the pathogen in sputum or tissue). Although 9 million new cases of active TB are still reported annually, the majority of infected individuals do not develop this form of the disease. It is estimated that up to one-third of the world's population (2 billion people) are infected with *M. tuberculosis*, yet they remain asymptomatic, defined as having latent TB (6). Epidemiological studies and modeling suggest that the majority of these individuals will control this latent infection lifelong, with only 5 to 10% reactivating infection to develop active TB during their lifetime.

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

outcomes of infection and to monitor vaccine efficacy.

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

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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).

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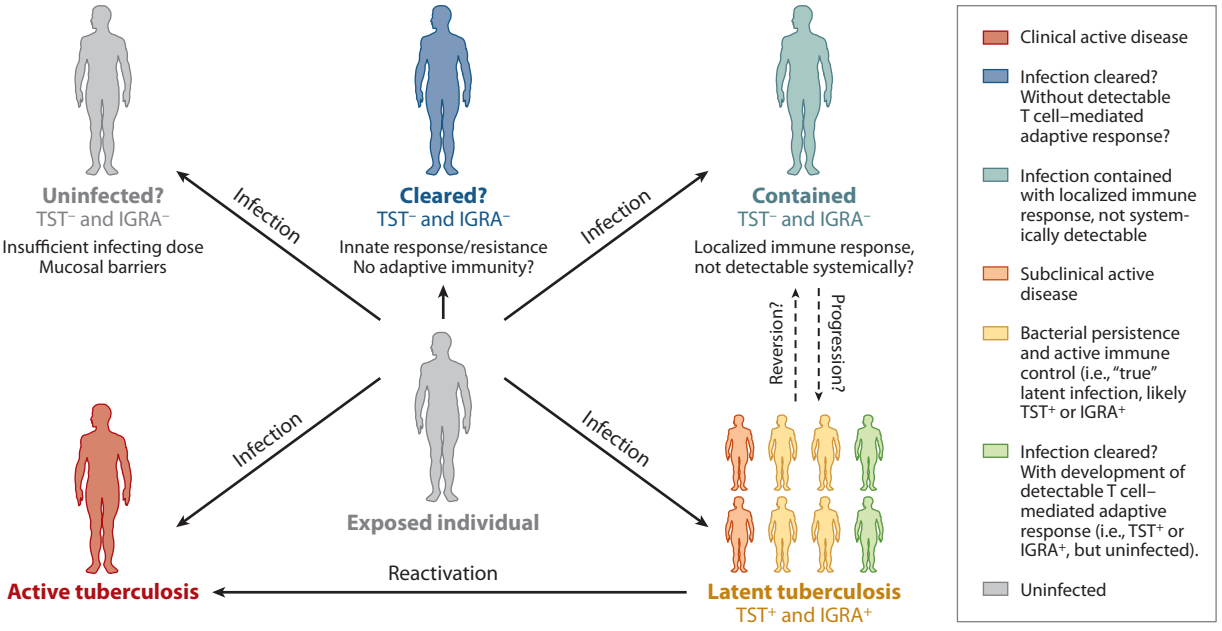

Figure 2

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 immunoassay after whole blood incubation (Qiagen) or by the enzyme-linked immunospot technique, which requires isolation of peripheral blood

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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 nonresponders 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

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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

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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

Figure 3

The transcriptional signature of active tuberculosis (TB) is dominated by an IFN-inducible blood transcriptional signature which is diminished upon treatment. (*a*) Hierarchical clustering analysis, plus additional statistical filtering, generated a gene tree (in green at the left of the figure) of 393 genes, and an expression profile (vertical columns) for each participant (healthy control and active TB). Each row of the heatmap represents an individual gene, and each column represents an individual participant. The relative abundance of transcripts is indicated by the color scale below the heatmaps (overabundance, *red*; underabundance, *blue*; median, *yellow*). The extent of the blood transcriptional signature correlated with the extent of radiographic disease measured by chest X-ray. This transcriptional signature of active TB is extinguished during treatment. Blood samples from active TB patients were taken 2 and 12 months after antimycobacterial drug treatment, and the transcriptional profiles at these times were compared with the baseline profiles. (*b*) Illustrative chest X-rays of the same infected individuals (patients 4 and 7) before, during, and after treatment, demonstrating that the diminution of the transcriptional signature reflects the clinical improvement in response to treatment. (*c*) The blood transcriptional signature of active TB is dominated by IFN-inducible genes (both type I and type II IFNs) as shown by Ingenuity Pathway Analysis (Reference 72).

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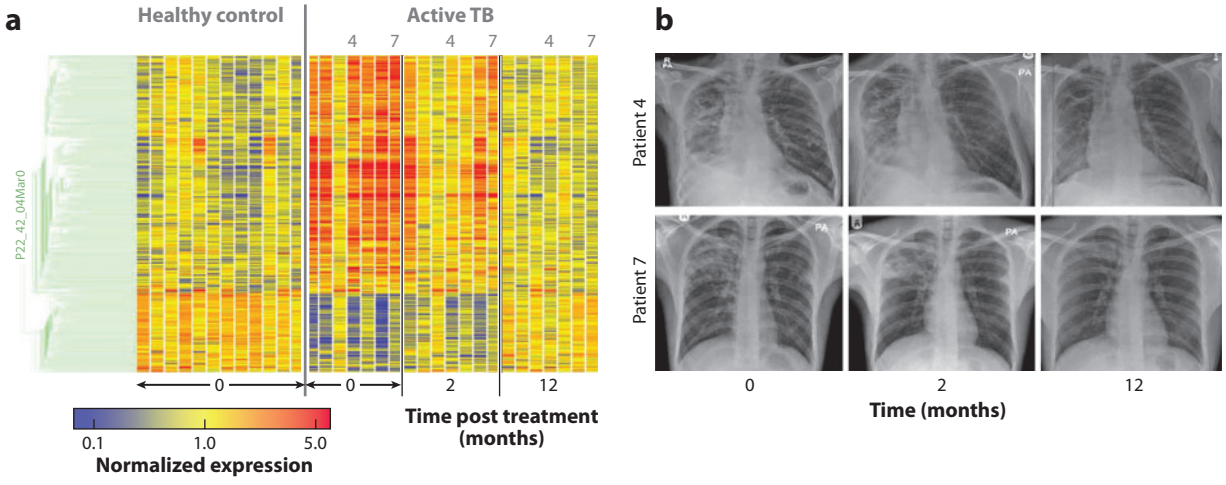
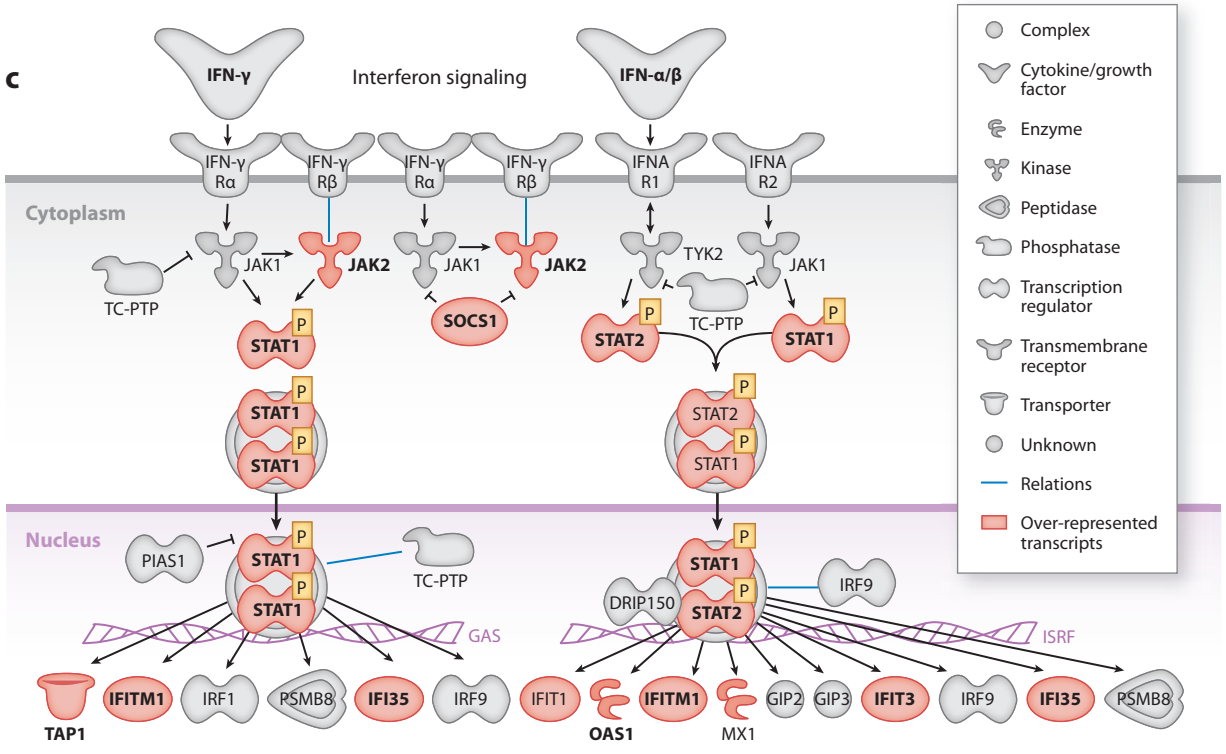
in 10–20% of them (72), demonstrating molecular heterogeneity of latent TB, which is consistent with the concept of a spectrum of responses to *M. tuberculosis* infection in latency. Whether this molecular heterogeneity reflects divergent clinical outcomes requires further work.

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





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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*

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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 mm^3, 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

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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 mm^3 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

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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

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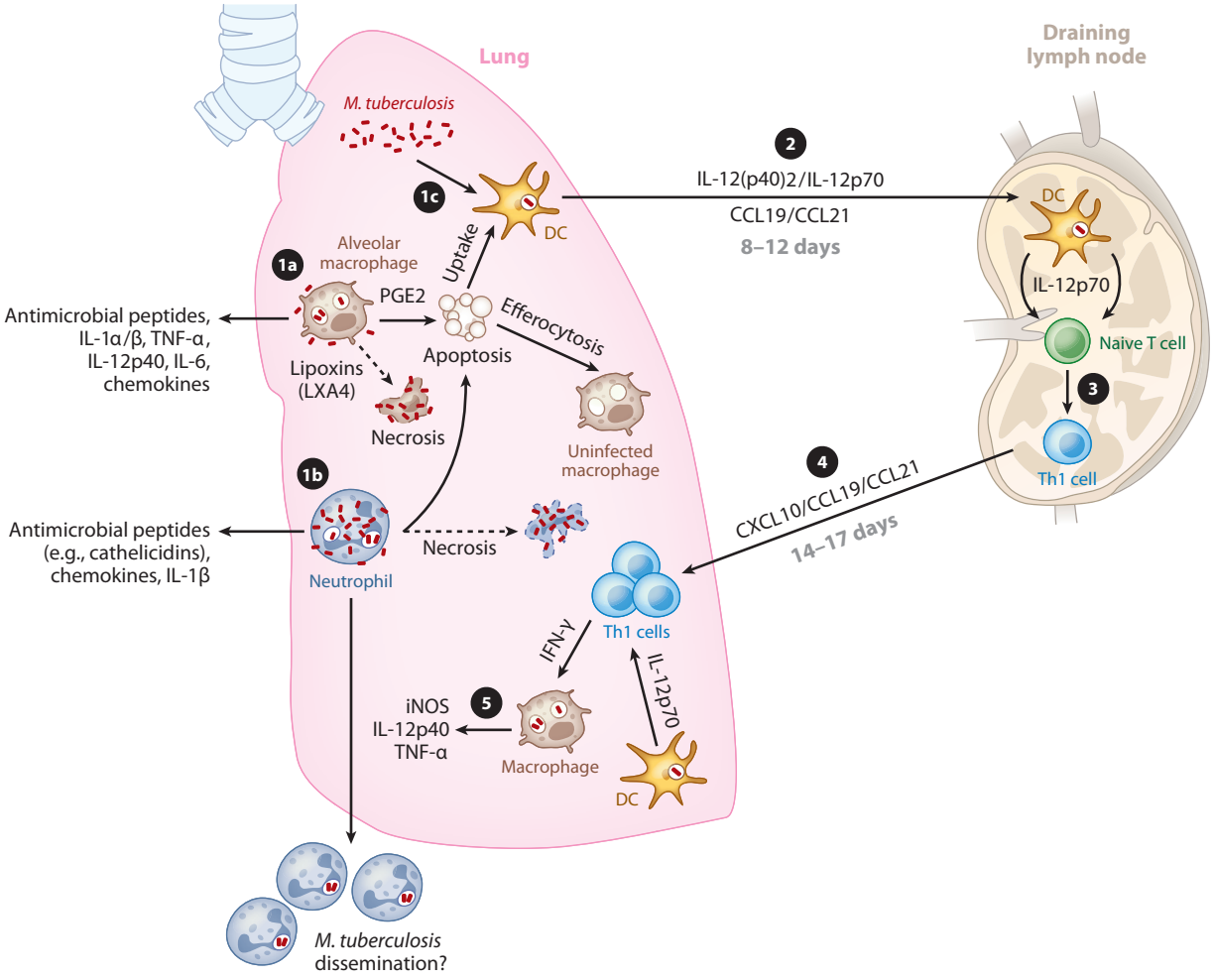

Figure 4

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.

nodes and occurs earlier in resistant C57BL/6 mice than in susceptible C3H mice, resulting in an earlier immune response in the C57BL/6 mice (134). This finding suggests that instead of only spreading infection, early 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).

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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

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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.

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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 fivefold 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 (*a*) by other host factors, including cytokines produced during the innate and adaptive immune response to *M. tuberculosis*, and (*b*) 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)D_3] 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)D_3] by 1 α-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

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D receptor (VDR) and VDBP are associated with an increased risk of TB (179).

$1,25(OH)D_3$ 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, $1,25(OH)D_3$ in IFN-γ-mediated restriction of intracellular growth of *M. tuberculosis* in vitro (119, 180). Ligation of TLR2 induces CYP27B1 and thus $1,25(OH)D_3$-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). A

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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 $\gamma\delta$ 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

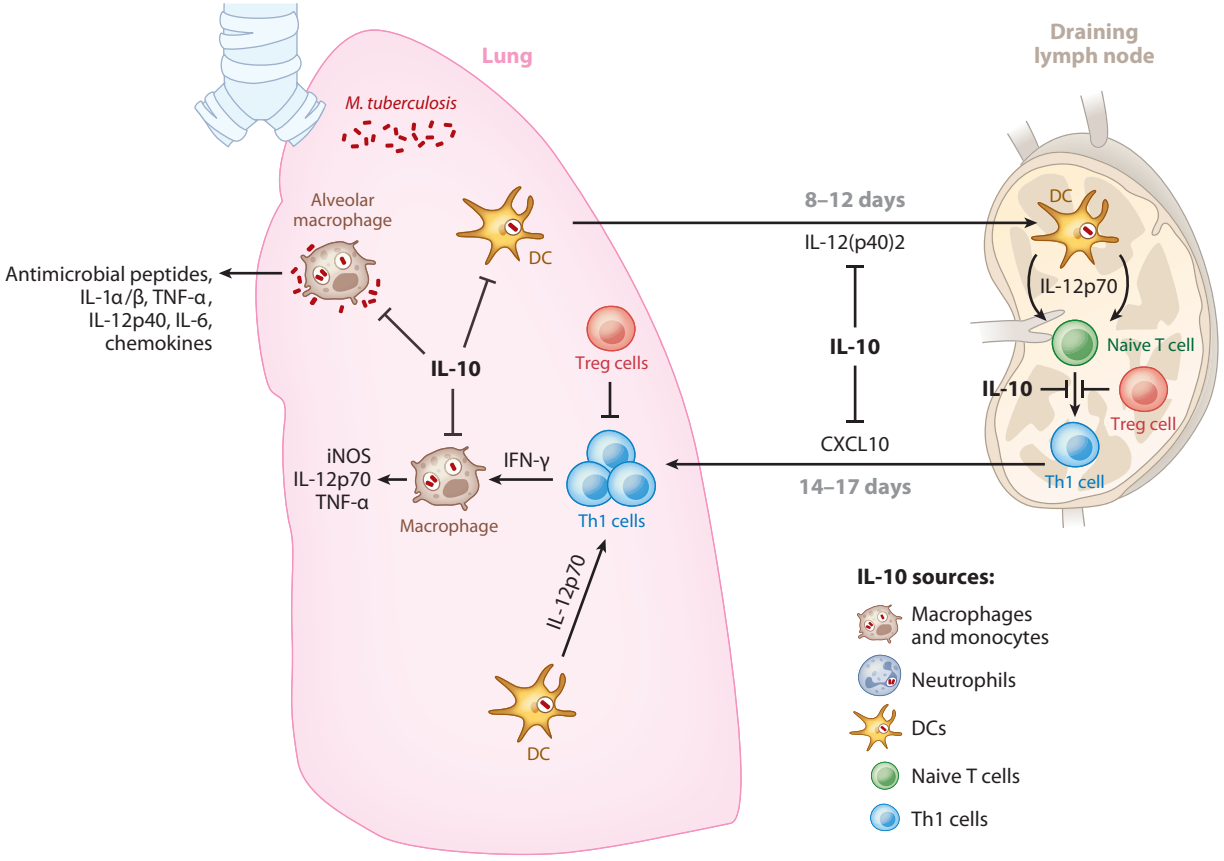

Figure 5

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.

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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

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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 FcγR 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 $CD4^+$ Th1 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 ($Il\text{-}12a^{-/-}$) 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, $IL\text{-}23p19^{-/-}$ 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

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otherwise healthy subjects to progressive infection with the vaccine strain *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 *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 *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 *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 *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 *Irf8* are susceptible to infection with intracellular pathogens, including *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 *Isg15*-deficient mice are highly susceptible to *M. tuberculosis* infection (243).

Although TB in children and adults generally constitutes two differing epidemiological and clinical forms of disease, an IL-12R-deficient patient presenting with disseminated TB as an adult (aged 33 years) may be

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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,

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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-γ 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-γ-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-γ-deficient mice showed increased numbers of IL-17-producing T cells following *M. bovis* BCG infection (283). These data demonstrated, first, that both IFN-γ- and IL-17-producing T cells are induced during mycobacterial infection and, second, that IFN-γ 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-γ 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 γδ 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-γ responses if IL-12p70 is available (232).

Following vaccination with a mycobacterial peptide, IL-17 is required for the accelerated recruitment of IFN-γ-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-γ 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

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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

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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 *Il10*$^{-/-}$ mice show increased trafficking to the draining lymph nodes in response to mycobacterial antigens, although effects on bacterial load were not assessed (311). *M. 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. 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. tuberculosis* survival.

Despite these proinflammatory responses in *Il10*$^{-/-}$ mice, only limited evidence supports a role for IL-10 in blocking host-mediated immunopathology during chronic *M. tuberculosis* infection (309). Our own work shows that abrogation of IL-10 signaling during *M. 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. 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-γ in the lungs at earlier stages post-infection, and these higher IL-10 concentrations contribute to the enhanced susceptibility of these mice to *M. 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-γ, and ultimately host survival.

Bone marrow–derived neutrophils infected in vitro (192) or neutrophils isolated from the lungs of mice challenged with *M. 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. 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. tuberculosis*–induced IL-10 in macrophages is dependent on endogenous levels of type I IFN and that the addition of IFN-β also greatly enhances the production of IL-10 in *M. 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. 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. 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. 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. tuberculosis* infection (308). However, *M. 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

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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).

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).

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*

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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, A. O'Garra, manuscript submitted).

It is becoming apparent that type I IFN increases susceptibility to *M. tuberculosis* infection via several mechanisms. The investigation of hypervirulent *M. tuberculosis* strains points to suppression of proinflammatory cytokines and Th1 immunity as playing a role (24, 99, 316, 325). The selective induction of type I IFN–associated genes and IFN-β occurs in macrophages infected with virulent but not avirulent *M. tuberculosis* with an inactive ESX-1 secretion system (325). This process of selective induction requires the downstream molecule TBK1 but not TRIF or RIP2, as shown recently (325, 328). Furthermore, type I IFN suppresses production of host-protective cytokines including IL-1 and IL-12 following *M. tuberculosis* infection, both in human macrophages (329) and in in vitro and in vivo mouse models (330; F. McNab, J. Ewbank, and A. O'Garra, manuscript submitted) (**Figure 6**). Induction of the immunosuppressive cytokine IL-10 by type I IFN appears to be an important mediator of these effects (330; F. McNab, J. Ewbank, and A. O'Garra, manuscript submitted) (**Figure 6**). Responsiveness to host-protective cytokines may also be impaired by type I IFN, as responsiveness to IFN-γ is reduced both in vitro and in vivo in the presence of high levels of type I IFN (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

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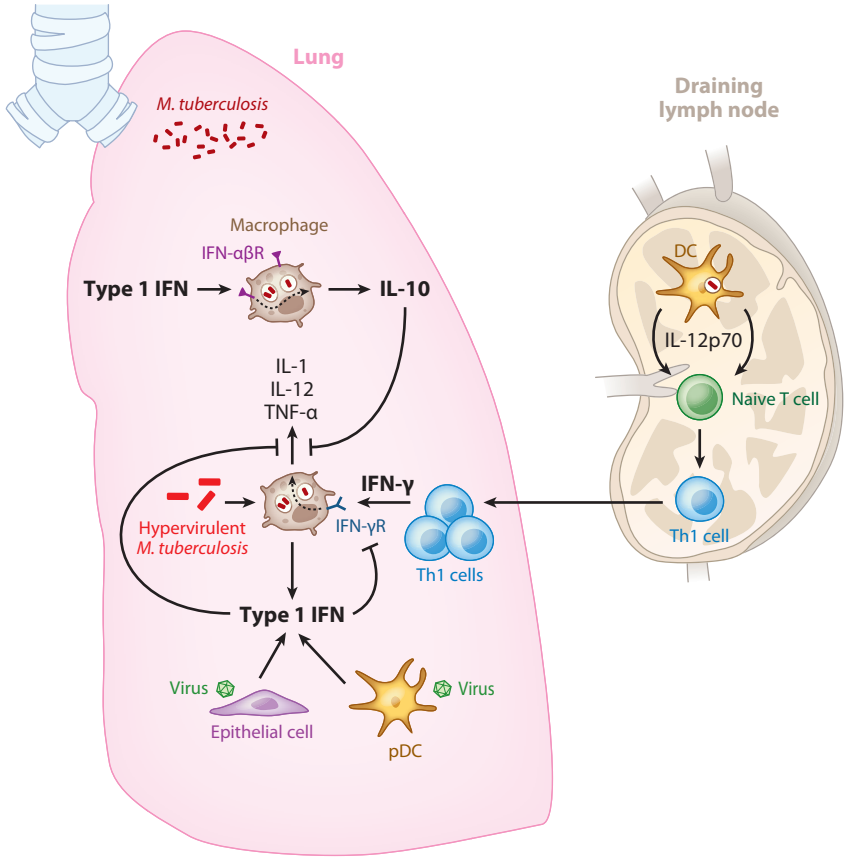

Figure 6

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

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(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 transinfection 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).

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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

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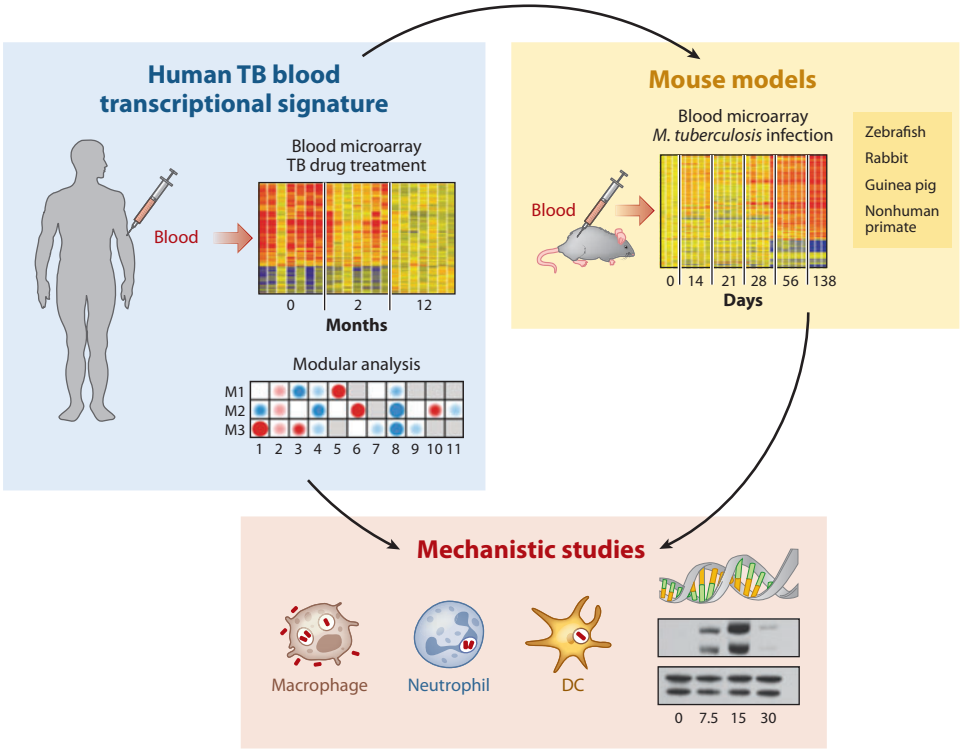

Figure 7

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 *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 *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 (372), body temperatures of the mice (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

1. WHO. 2012. *World Health Organization: Tuberculosis—Global Facts 2011/2102*. Geneva: WHO Stop TB Dep. **http://www.who.int/tb/publications/2011/factsheet_tb_2011.pdf**
2. Young DB, Perkins MD, Duncan K, Barry CE 3rd. 2008. Confronting the scientific obstacles to global control of tuberculosis. *J. Clin. Investig.* 118:1255–65
3. Koch R. 1882. The etiology of tuberculosis. *Berl. Klin. Wochenschr.* 15:221–30
4. Harisinghani MG, McLoud TC, Shepard JA, Ko JP, Shroff MM, Mueller PR. 2000. Tuberculosis from head to toe. *Radiographics* 20:449–70
5. CCDC. 2007. *Center for Communicable Disease Control and Prevention. Reported Tuberculosis in the United States, 2007*. Atlanta, GA: US Dep. Health Hum. Serv.
6. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. 1999. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA* 282:677–86
7. Comstock GW, Livesay VT, Woolpert SF. 1974. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am. J. Epidemiol.* 99:131–38
8. Vynnycky E, Fine PE. 2000. Lifetime risks, incubation period, and serial interval of tuberculosis. *Am. J. Epidemiol.* 152:247–63
9. Kaufmann SH. 2012. Tuberculosis vaccine development: strength lies in tenacity. *Trends Immunol.* 33:373–79
10. McShane H, Jacobs WR, Fine PE, Reed SG, McMurray DN, et al. 2012. BCG: myths, realities, and the need for alternative vaccine strategies. *Tuberculosis* 92:283–88
11. Cooper AM. 2009. Cell-mediated immune responses in tuberculosis. *Annu. Rev. Immunol.* 27:393–422
12. Flynn JL, Chan J. 2001. Immunology of tuberculosis. *Annu. Rev. Immunol.* 19:93–129
13. North RJ, Jung YJ. 2004. Immunity to tuberculosis. *Annu. Rev. Immunol.* 22:599–623
14. Alcais A, Fieschi C, Abel L, Casanova JL. 2005. Tuberculosis in children and adults: two distinct genetic diseases. *J. Exp. Med.* 202:1617–21
15. Alcais A, Quintana-Murci L, Thaler DS, Schurr E, Abel L, Casanova JL. 2010. Life-threatening infectious diseases of childhood: single-gene inborn errors of immunity? *Ann. N.Y. Acad. Sci.* 1214:18–33
16. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, et al. 1995. Tumor necrosis factor-α is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity* 2:561–72

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17. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, et al. 2001. Tuberculosis associated with infliximab, a tumor necrosis factor α–neutralizing agent. *N. Engl. J. Med.* 345:1098–104
18. Barry CE 3rd, Boshoff HI, Dartois V, Dick T, Ehrt S, et al. 2009. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat. Rev. Microbiol.* 7:845–55
19. Kagina BM, Abel B, Scriba TJ, Hughes EJ, Keyser A, et al. 2010. Specific T cell frequency and cytokine expression profile do not correlate with protection against tuberculosis after bacillus Calmette-Guerin vaccination of newborns. *Am. J. Respir. Crit. Care Med.* 182:1073–79
20. Apt A, Kramnik I. 2009. Man and mouse TB: contradictions and solutions. *Tuberculosis* 89:195–98
21. Fortin A, Abel L, Casanova JL, Gros P. 2007. Host genetics of mycobacterial diseases in mice and men: forward genetic studies of BCG-osis and tuberculosis. *Annu. Rev. Genomics Hum. Genet.* 8:163–92
22. Gagneux S. 2012. Host-pathogen coevolution in human tuberculosis. *Philos. Trans. R. Soc. Lond. Ser. B* 367:850–59
23. Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong BC, et al. 2006. Variable host-pathogen compatibility in *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. USA* 103:2869–73
24. Manca C, Tsenova L, Bergtold A, Freeman S, Tovey M, et al. 2001. Virulence of a *Mycobacterium tuberculosis* clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of IFN-α/β. *Proc. Natl. Acad. Sci. USA* 98:5752–57
25. Nicol MP, Wilkinson RJ. 2008. The clinical consequences of strain diversity in *Mycobacterium tuberculosis*. *Trans. R. Soc. Trop. Med. Hyg.* 102:955–65
26. de Jong BC, Hill PC, Aiken A, Awine T, Antonio M, et al. 2008. Progression to active tuberculosis, but not transmission, varies by *Mycobacterium tuberculosis* lineage in The Gambia. *J. Infect. Dis.* 198:1037–43
27. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. 2003. Tuberculosis. *Lancet* 362:887–99
28. Brandli O. 1998. The clinical presentation of tuberculosis. *Respir. Int. Rev. Thorac. Dis.* 65:97–105
29. Wyngarden JB. 1988. *Cecil Textbook of Medicine.* Philadelphia: W.B. Saunders
30. Storla DG, Yimer S, Bjune GA. 2008. A systematic review of delay in the diagnosis and treatment of tuberculosis. *BMC Public Health* 8:15
31. Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, et al. 2006. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infect. Dis.* 6:570–81
32. Pfyffer GE, Cieslak C, Welscher HM, Kissling P, Rusch-Gerdes S. 1997. Rapid detection of mycobacteria in clinical specimens by using the automated BACTEC 9000 MB system and comparison with radiometric and solid-culture systems. *J. Clin. Microbiol.* 35:2229–34
33. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, et al. 2010. Rapid molecular detection of tuberculosis and rifampin resistance. *N. Engl. J. Med.* 363:1005–15
34. Stop TB Partnersh. 2010. *The Global Plan to Stop TB 2011–2015*. Geneva: WHO
35. Schoch OD, Rieder P, Tueller C, Altpeter E, Zellweger JP, et al. 2007. Diagnostic yield of sputum, induced sputum, and bronchoscopy after radiologic tuberculosis screening. *Am. J. Respir. Crit. Care Med.* 175:80–86
36. Millen SJ, Uys PW, Hargrove J, van Helden PD, Williams BG. 2008. The effect of diagnostic delays on the drop-out rate and the total delay to diagnosis of tuberculosis. *PLoS ONE* 3:e1933
37. Fox W, Ellard GA, Mitchison DA. 1999. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council Tuberculosis Units, 1946–1986, with relevant subsequent publications. *Int. J. Tuberc. Lung Dis.* 3(Suppl.):S231–79
38. Natl. Inst. Health Clin. Excell., Natl. Collab. Cent. Chronic Cond. 2006. *Tuberculosis: Clinical Diagnosis and Management of Tuberculosis, and Measures for Its Prevention and Control*. London: R. Coll. Phys.
39. Med. Res. Counc. 1988. Management and outcome of pulmonary tuberculosis in adults notified in England and Wales in 1983. Medical Research Council Tuberculosis and Chest Diseases Unit. *Thorax* 43:591–98
40. Mitchison DA. 1993. Assessment of new sterilizing drugs for treating pulmonary tuberculosis by culture at 2 months. *Am. Rev. Respir. Dis.* 147:1062–63
41. Horne DJ, Royce SE, Gooze L, Narita M, Hopewell PC, et al. 2010. Sputum monitoring during tuberculosis treatment for predicting outcome: systematic review and meta-analysis. *Lancet Infect. Dis.* 10:387–94

42. Walzl G, Ronacher K, Hanekom W, Scriba TJ, Zumla A. 2011. Immunological biomarkers of tuberculosis. *Nat. Rev. Immunol.* 11:343–54
43. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, et al. 2003. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch. Intern. Med.* 163:1009–21
44. Gomez JE, McKinney JD. 2004. *M. tuberculosis* persistence, latency, and drug tolerance. *Tuberculosis* 84:29–44
45. Lillebaek T, Dirksen A, Vynnycky E, Baess I, Thomsen VO, Andersen AB. 2003. Stability of DNA patterns and evidence of *Mycobacterium tuberculosis* reactivation occurring decades after the initial infection. *J. Infect. Dis.* 188:1032–39
46. Chan J, Flynn J. 2004. The immunological aspects of latency in tuberculosis. *Clin. Immunol.* 110:2–12
47. Vandiviere HM, Loring WE, Melvin I, Willis S. 1956. The treated pulmonary lesion and its tubercle bacillus. II. The death and resurrection. *Am. J. Med. Sci.* 232:30–37
48. Lin PL, Flynn JL. 2010. Understanding latent tuberculosis: a moving target. *J. Immunol.* 185:15–22
49. Young DB, Gideon HP, Wilkinson RJ. 2009. Eliminating latent tuberculosis. *Trends Microbiol.* 17:183–88
50. Pirquet V. 1907. Frequency of tuberculosis in childhood. *JAMA* 52:675–78
51. Mantoux MC. 1912. La voie intradermique en tubercalinotherapie. *Presse Med.* 20:146–48
52. Reichman LB. 1979. Tuberculin skin testing. The state of the art. *Chest* 76:764–70
53. Holden M, Dubin MR, Diamond PH. 1971. Frequency of negative intermediate-strength tuberculin sensitivity in patients with active tuberculosis. *N. Engl. J. Med.* 285:1506–9
54. Richeldi L. 2006. An update on the diagnosis of tuberculosis infection. *Am. J. Respir. Crit. Care Med.* 174:736–42
55. Andersen P, Doherty TM. 2005. The success and failure of BCG—implications for a novel tuberculosis vaccine. *Nat. Rev. Microbiol.* 3:656–62
56. Horsburgh CR Jr. 2004. Priorities for the treatment of latent tuberculosis infection in the United States. *N. Engl. J. Med.* 350:2060–67
57. Van Rhijn I, Nguyen TK, Michel A, Cooper D, Govaerts M, et al. 2009. Low cross-reactivity of T-cell responses against lipids from *Mycobacterium bovis* and *M. avium paratuberculosis* during natural infection. *Eur. J. Immunol.* 39:3031–41
58. Harboe M, Oettinger T, Wiker HG, Rosenkrands I, Andersen P. 1996. Evidence for occurrence of the ESAT-6 protein in *Mycobacterium tuberculosis* and virulent *Mycobacterium bovis* and for its absence in *Mycobacterium bovis* BCG. *Infect. Immun.* 64:16–22
59. Jereb J, Etkind SC, Joglar OT, Moore M, Taylor Z. 2003. Tuberculosis contact investigations: outcomes in selected areas of the United States, 1999. *Int. J. Tuberc. Lung Dis.* 7:S384–90
60. Marks SM, Taylor Z, Qualls NL, Shrestha-Kuwahara RJ, Wilce MA, Nguyen CH. 2000. Outcomes of contact investigations of infectious tuberculosis patients. *Am. J. Respir. Crit. Care Med.* 162:2033–38
61. Styblo K. 1980. Recent advances in epidemiological research in tuberculosis. *Adv. Tuberc. Res.* 20:1–63
62. Cobat A, Gallant CJ, Simkin L, Black GF, Stanley K, et al. 2010. High heritability of antimycobacterial immunity in an area of hyperendemicity for tuberculosis disease. *J. Infect. Dis.* 201:15–19
63. Cobat A, Gallant CJ, Simkin L, Black GF, Stanley K, et al. 2009. Two loci control tuberculin skin test reactivity in an area hyperendemic for tuberculosis. *J. Exp. Med.* 206:2583–91
64. Medlar EM. 1948. The pathogenesis of minimal pulmonary tuberculosis; a study of 1,225 necropsies in cases of sudden and unexpected death. *Am. Rev. Tuberc.* 58:583–611
65. Capuano SV, Croix DA, Pawar S, Zinovik A, Myers A, et al. 2003. Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. *Infect. Immun.* 71:5831–44
66. Fennelly KP, Jones-Lopez EC, Ayakaka I, Kim S, Menyha H, et al. 2012. Variability of infectious aerosols produced during coughing by patients with pulmonary tuberculosis. *Am. J. Respir. Crit. Care Med.* 186:450–57
67. Escombe AR, Oeser C, Gilman RH, Navincopa M, Ticona E, et al. 2007. The detection of airborne transmission of tuberculosis from HIV-infected patients, using an in vivo air sampling model. *Clin. Infect. Dis.* 44:1349–57
68. Mack U, Migliori GB, Sester M, Rieder HL, Ehlers S, et al. 2009. LTBI: latent tuberculosis infection or lasting immune responses to *M. tuberculosis*? A TBNET consensus statement. *Eur. Respir. J.* 33:956–73

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69. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, et al. 1999. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 286:531–37
70. Bonnefoi H, Underhill C, Iggo R, Cameron D. 2009. Predictive signatures for chemotherapy sensitivity in breast cancer: are they ready for use in the clinic? *Eur. J. Cancer* 45:1733–43
71. Pascual V, Chaussabel D, Banchereau J. 2010. A genomic approach to human autoimmune diseases. *Annu. Rev. Immunol.* 28:535–71
72. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, et al. 2010. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* 466:973–77
73. Bloom CI, Graham CM, Berry MP, Wilkinson KA, Oni T, et al. 2012. Detectable changes in the blood transcriptome are present after two weeks of antituberculosis therapy. *PLoS ONE* 7:e46191
74. Ernst JD, Hanekom W, Hawn T, Kampmann B, Rengarajan J. 2012. Meeting report: The International Conference on Human Immunity to Tuberculosis. *Tuberculosis* 92:440–44
75. Maertzdorf J, Repsilber D, Parida SK, Stanley K, Roberts T, et al. 2011. Human gene expression profiles of susceptibility and resistance in tuberculosis. *Genes Immun.* 12:15–22
76. Ottenhoff TH, Dass RH, Yang N, Zhang MM, Wong HE, et al. 2012. Genome-wide expression profiling identifies type 1 interferon response pathways in active tuberculosis. *PLoS ONE* 7:e45839
77. Cliff JM, Lee JS, Constantinou N, Cho JE, Clark TG, et al. 2013. Distinct phases of blood gene expression pattern through tuberculosis treatment reflect modulation of the humoral immune response. *J. Infect. Dis.* 207:18–29
78. Jacobsen M, Repsilber D, Kleinsteuber K, Gutschmidt A, Schommer-Leitner S, et al. 2011. Suppressor of cytokine signaling-3 is affected in T-cells from tuberculosis TB patients. *Clin. Microbiol. Infect.* 17:1323–31
79. Chaussabel D, Allman W, Mejias A, Chung W, Bennett L, et al. 2005. Analysis of significance patterns identifies ubiquitous and disease-specific gene-expression signatures in patient peripheral blood leukocytes. *Ann. N.Y. Acad. Sci.* 1062:146–54
80. Chaussabel D, Quinn C, Shen J, Patel P, Glaser C, et al. 2008. A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus. *Immunity* 29:150–64
81. Koth LL, Solberg OD, Peng JC, Bhakta NR, Nguyen CP, Woodruff PG. 2011. Sarcoidosis blood transcriptome reflects lung inflammation and overlaps with tuberculosis. *Am. J. Respir. Crit. Care Med.* 184:1153–63
82. Maertzdorf J, Weiner J 3rd, Mollenkopf HJ, Network T, Bauer T, et al. 2012. Common patterns and disease-related signatures in tuberculosis and sarcoidosis. *Proc. Natl. Acad. Sci. USA* 109:7853–58
83. Iannuzzi MC, Rybicki BA, Teirstein AS. 2007. Sarcoidosis. *N. Engl. J. Med.* 357:2153–65
84. Medina E, North RJ. 1998. Resistance ranking of some common inbred mouse strains to *Mycobacterium tuberculosis* and relationship to major histocompatibility complex haplotype and *Nramp1* genotype. *Immunology* 93:270–74
85. Via LE, Lin PL, Ray SM, Carrillo J, Allen SS, et al. 2008. Tuberculous granulomas are hypoxic in guinea pigs, rabbits, and nonhuman primates. *Infect. Immun.* 76:2333–40
86. McMurray DN. 2001. Disease model: pulmonary tuberculosis. *Trends Mol. Med.* 7:135–37
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*. *J. Exp. Med.* 186:39–45
88. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. 1993. An essential role for interferon γ in resistance to *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 178:2249–54
89. Orme IM, Andersen P, Boom WH. 1993. T cell response to *Mycobacterium tuberculosis*. *J. Infect. Dis.* 167:1481–97
90. Geldmacher C, Ngwenyama N, Schuetz A, Petrovas C, Reither K, et al. 2010. Preferential infection and depletion of *Mycobacterium tuberculosis*-specific CD4 T cells after HIV-1 infection. *J. Exp. Med.* 207:2869–81
91. Geldmacher C, Schuetz A, Ngwenyama N, Casazza JP, Sanga E, et al. 2008. Early depletion of *Mycobacterium tuberculosis*-specific T helper 1 cell responses after HIV-1 infection. *J. Infect. Dis.* 198:1590–98
92. Chackerian AA, Behar SM. 2003. Susceptibility to *Mycobacterium tuberculosis*: lessons from inbred strains of mice. *Tuberculosis* 83:279–85

93. Marquis JF, Nantel A, LaCourse R, Ryan L, North RJ, Gros P. 2008. Fibrotic response as a distinguishing feature of resistance and susceptibility to pulmonary infection with *Mycobacterium tuberculosis* in mice. *Infect. Immun.* 76:78–88
94. Mitsos LM, Cardon LR, Fortin A, Ryan L, LaCourse R, et al. 2000. Genetic control of susceptibility to infection with *Mycobacterium tuberculosis* in mice. *Genes Immun.* 1:467–77
95. Keller C, Hoffmann R, Lang R, Brandau S, Hermann C, Ehlers S. 2006. Genetically determined susceptibility to tuberculosis in mice causally involves accelerated and enhanced recruitment of granulocytes. *Infect. Immun.* 74:4295–309
96. Manabe YC, Kesavan AK, Lopez-Molina J, Hatem CL, Brooks M, et al. 2008. The aerosol rabbit model of TB latency, reactivation and immune reconstitution inflammatory syndrome. *Tuberculosis* 88:187–96
97. Subbian S, Tsenova L, O'Brien P, Yang G, Koo MS, et al. 2011. Phosphodiesterase-4 inhibition combined with isoniazid treatment of rabbits with pulmonary tuberculosis reduces macrophage activation and lung pathology. *Am. J. Pathol.* 179:289–301
98. Subbian S, Tsenova L, Yang G, O'Brien P, Parsons S, et al. 2011. Chronic pulmonary cavitary tuberculosis in rabbits: a failed host immune response. *Open Biol.* 1:110016
99. Manca C, Tsenova L, Freeman S, Barczak AK, Tovey M, et al. 2005. Hypervirulent *M. tuberculosis* W/Beijing strains upregulate type I IFNs and increase expression of negative regulators of the Jak-Stat pathway. *J. Interferon Cytokine Res.* 25:694–701
100. Lin PL, Rodgers M, Smith L, Bigbee M, Myers A, et al. 2009. Quantitative comparison of active and latent tuberculosis in the cynomolgus macaque model. *Infect. Immun.* 77:4631–42
101. Russell DG, Cardona PJ, Kim MJ, Allain S, Altare F. 2009. Foamy macrophages and the progression of the human tuberculosis granuloma. *Nat. Immunol.* 10:943–48
102. Peters W, Ernst JD. 2003. Mechanisms of cell recruitment in the immune response to *Mycobacterium tuberculosis*. *Microbes Infect.* 5:151–58
103. Egen JG, Rothfuchs AG, Feng CG, Horwitz MA, Sher A, Germain RN. 2011. Intravital imaging reveals limited antigen presentation and T cell effector function in mycobacterial granulomas. *Immunity* 34:807–19
104. Ulrichs T, Kosmiadi GA, Trusov V, Jorg S, Pradl L, et al. 2004. Human tuberculous granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defence in the lung. *J. Pathol.* 204:217–28
105. Hernández-Pando R, Jeyanathan M, Mengistu G, Aguilar D, Orozco H, et al. 2000. Persistence of DNA from *Mycobacterium tuberculosis* in superficially normal lung tissue during latent infection. *Lancet* 356:2133–38
106. Tsai MC, Chakravarty S, Zhu G, Xu J, Tanaka K, et al. 2006. Characterization of the tuberculous granuloma in murine and human lungs: cellular composition and relative tissue oxygen tension. *Cell. Microbiol.* 8:218–32
107. Reece ST, Loddenkemper C, Askew DJ, Zedler U, Schommer-Leitner S, et al. 2010. Serine protease activity contributes to control of *Mycobacterium tuberculosis* in hypoxic lung granulomas in mice. *J. Clin. Investig.* 120:3365–76
108. Saunders BM, Orme IM, Basaraba R. 2008. Immunopathology of tuberculosis. In *Handbook of Tuberculosis: Immunology and Cell Biology*, ed. SHE Kaufmann, WJ Britton, pp. 245–78. Germany: Wiley-VCH
109. Davis JM, Ramakrishnan L. 2009. The role of the granuloma in expansion and dissemination of early tuberculous infection. *Cell* 136:37–49
110. Ramakrishnan L. 2012. Revisiting the role of the granuloma in tuberculosis. *Nat. Rev. Immunol.* 12:352–66
111. Tobin DM, Vary JC Jr, Ray JP, Walsh GS, Dunstan SJ, et al. 2010. The *lta4h* locus modulates susceptibility to mycobacterial infection in zebrafish and humans. *Cell* 140:717–30
112. Garton NJ, Waddell SJ, Sherratt AL, Lee SM, Smith RJ, et al. 2008. Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum. *PLoS Med.* 5:e75
113. Schlesinger LS. 1996. Entry of *Mycobacterium tuberculosis* into mononuclear phagocytes. *Curr. Top. Microbiol. Immunol.* 215:71–96

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114. Eruslanov EB, Lyadova IV, Kondratieva TK, Majorov KB, Scheglov IV, et al. 2005. Neutrophil responses to *Mycobacterium tuberculosis* infection in genetically susceptible and resistant mice. *Infect. Immun.* 73:1744–53
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
116. Wolf AJ, Linas B, Trevejo-Nuñez GJ, Kincaid E, Tamura T, et al. 2007. *Mycobacterium tuberculosis* infects dendritic cells with high frequency and impairs their function in vivo. *J. Immunol.* 179:2509–19
117. Lowe DM, Redford PS, Wilkinson RJ, O'Garra A, Martineau AR. 2012. Neutrophils in tuberculosis: friend or foe? *Trends Immunol.* 33:14–25
118. Castañeda-Delgado J, Hernández-Pando R, Serrano CJ, Aguilar-Leon D, León-Contreras J, et al. 2010. Kinetics and cellular sources of cathelicidin during the course of experimental latent tuberculous infection and progressive pulmonary tuberculosis. *Clin. Exp. Immunol.* 161:542–50
119. Fabri M, Stenger S, Shin DM, Yuk JM, Liu PT, et al. 2011. Vitamin D is required for IFN-γ-mediated antimicrobial activity of human macrophages. *Sci. Transl. Med.* 3:104ra2
120. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, et al. 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 311:1770–73
121. Martineau AR, Honecker FU, Wilkinson RJ, Griffiths CJ. 2007. Vitamin D in the treatment of pulmonary tuberculosis. *J. Steroid Biochem. Mol. Biol.* 103:793–98
122. Martineau AR, Wilkinson KA, Newton SM, Floto RA, Norman AW, et al. 2007. IFN-γ- and TNF-independent vitamin D-inducible human suppression of mycobacteria: the role of cathelicidin LL-37. *J. Immunol.* 178:7190–98
123. Behar SM, Martin CJ, Booty MG, Nishimura T, Zhao X, et al. 2011. Apoptosis is an innate defense function of macrophages against *Mycobacterium tuberculosis*. *Mucosal Immunol.* 4:279–87
124. Cooper AM, Mayer-Barber KD, Sher A. 2011. Role of innate cytokines in mycobacterial infection. *Mucosal Immunol.* 4:252–60
125. Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG, Van Crevel R. 2011. Innate immune recognition of *Mycobacterium tuberculosis*. *Clin. Dev. Immunol.* 2011:405310
126. Armstrong JA, Hart PD. 1971. Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. *J. Exp. Med.* 134:713–40
127. Colombo MI, Gutierrez MG, Romano PS. 2006. The two faces of autophagy: *Coxiella* and *Mycobacterium*. *Autophagy* 2:162–64
128. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. 2004. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* 119:753–66
129. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, et al. 1994. Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* 263:678–81
130. Armstrong JA, Hart PD. 1975. Phagosome-lysosome interactions in cultured macrophages infected with virulent tubercle bacilli. Reversal of the usual nonfusion pattern and observations on bacterial survival. *J. Exp. Med.* 142:1–16
131. Pieters J. 2008. *Mycobacterium tuberculosis* and the macrophage: maintaining a balance. *Cell Host Microbe* 3:399–407
132. Behar SM, Divangahi M, Remold HG. 2010. Evasion of innate immunity by *Mycobacterium tuberculosis*: is death an exit strategy? *Nat. Rev. Microbiol.* 8:668–74
133. Shafiani S, Tucker-Heard G, Kariyone A, Takatsu K, Urdahl KB. 2010. Pathogen-specific regulatory T cells delay the arrival of effector T cells in the lung during early tuberculosis. *J. Exp. Med.* 207:1409–20
134. Chackerian AA, Alt JM, Perera TV, Dascher CC, Behar SM. 2002. Dissemination of *Mycobacterium tuberculosis* is influenced by host factors and precedes the initiation of T-cell immunity. *Infect. Immun.* 70:4501–9
135. Keane J, Remold HG, Kornfeld H. 2000. Virulent *Mycobacterium tuberculosis* strains evade apoptosis of infected alveolar macrophages. *J. Immunol.* 164:2016–20
136. Kelly DM, ten Bokum AM, O'Leary SM, O'Sullivan MP, Keane J. 2008. Bystander macrophage apoptosis after *Mycobacterium tuberculosis* H37Ra infection. *Infect. Immun.* 76:351–60

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137. Hinchey J, Lee S, Jeon BY, Basaraba RJ, Venkataswamy MM, et al. 2007. Enhanced priming of adaptive immunity by a proapoptotic mutant of *Mycobacterium tuberculosis*. *J. Clin. Investig.* 117:2279–88
138. Velmurugan K, Chen B, Miller JL, Azogue S, Gurses S, et al. 2007. *Mycobacterium tuberculosis nuoG* is a virulence gene that inhibits apoptosis of infected host cells. *PLoS Pathog.* 3:e110
139. Martin CJ, Booty MG, Rosebrock TR, Nunes-Alves C, Desjardins DM, et al. 2012. Efferocytosis is an innate antibacterial mechanism. *Cell Host Microbe* 12:289–300
140. Liao X, Sluimer JC, Wang Y, Subramanian M, Brown K, et al. 2012. Macrophage autophagy plays a protective role in advanced atherosclerosis. *Cell Metab.* 15:545–53
141. Bafica A, Scanga CA, Serhan C, Machado F, White S, et al. 2005. Host control of *Mycobacterium tuberculosis* is regulated by 5-lipoxygenase-dependent lipoxin production. *J. Clin. Investig.* 115:1601–6
142. Chen M, Divangahi M, Gan H, Shin DS, Hong S, et al. 2008. Lipid mediators in innate immunity against tuberculosis: opposing roles of PGE2 and LXA4 in the induction of macrophage death. *J. Exp. Med.* 205:2791–801
143. Divangahi M, Desjardins D, Nunes-Alves C, Remold HG, Behar SM. 2010. Eicosanoid pathways regulate adaptive immunity to *Mycobacterium tuberculosis*. *Nat. Immunol.* 11:751–58
144. Abadie V, Badell E, Douillard P, Ensergueix D, Leenen PJ, et al. 2005. Neutrophils rapidly migrate via lymphatics after *Mycobacterium bovis* BCG intradermal vaccination and shuttle live bacilli to the draining lymph nodes. *Blood* 106:1843–50
145. Sutherland JS, Jeffries DJ, Donkor S, Walther B, Hill PC, et al. 2009. High granulocyte/lymphocyte ratio and paucity of NKT cells defines TB disease in a TB-endemic setting. *Tuberculosis* 89:398–404
146. Seiler P, Aichele P, Bandermann S, Hauser AE, Lu B, et al. 2003. Early granuloma formation after aerosol *Mycobacterium tuberculosis* infection is regulated by neutrophils via CXCR3-signaling chemokines. *Eur. J. Immunol.* 33:2676–86
147. Bandara A, Bremner S, Barker R, Packe G, Griffiths C, Martineau A. 2008. Neutrophilia in tuberculosis. *Thorax* 63(Suppl.):A111–17
148. Barnes PF, Leedom JM, Chan LS, Wong SF, Shah J, et al. 1988. Predictors of short-term prognosis in patients with pulmonary tuberculosis. *J. Infect. Dis.* 158:366–71
149. Blomgran R, Ernst JD. 2011. Lung neutrophils facilitate activation of naive antigen-specific $CD4^+$ T cells during *Mycobacterium tuberculosis* infection. *J. Immunol.* 186:7110–19
150. Blomgran R, Desvignes L, Briken V, Ernst JD. 2012. *Mycobacterium tuberculosis* inhibits neutrophil apoptosis, leading to delayed activation of naive CD4 T cells. *Cell Host Microbe* 11:81–90
151. Zhang X, Majlessi L, Deriaud E, Leclerc C, Lo-Man R. 2009. Coactivation of Syk kinase and MyD88 adaptor protein pathways by bacteria promotes regulatory properties of neutrophils. *Immunity* 31:761–71
152. Cruz A, Fraga AG, Fountain JJ, Rangel-Moreno J, Torrado E, et al. 2010. Pathological role of interleukin 17 in mice subjected to repeated BCG vaccination after infection with *Mycobacterium tuberculosis*. *J. Exp. Med.* 207:1609–16
153. Redford PS, Boonstra A, Read S, Pitt J, Graham C, et al. 2010. Enhanced protection to *Mycobacterium tuberculosis* infection in IL-10-deficient mice is accompanied by early and enhanced Th1 responses in the lung. *Eur. J. Immunol.* 40:2200–10
154. Nandi B, Behar SM. 2011. Regulation of neutrophils by interferon-γ limits lung inflammation during tuberculosis infection. *J. Exp. Med.* 208:2251–62
155. Bean AG, Roach DR, Briscoe H, France MP, Korner H, et al. 1999. Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol *Mycobacterium tuberculosis* infection, which is not compensated for by lymphotoxin. *J. Immunol.* 162:3504–11
156. Kaneko H, Yamada H, Mizuno S, Udagawa T, Kazumi Y, et al. 1999. Role of tumor necrosis factor-α in Mycobacterium-induced granuloma formation in tumor necrosis factor-α-deficient mice. *Lab. Investig.* 79:379–86
157. Botha T, Ryffel B. 2003. Reactivation of latent tuberculosis infection in TNF-deficient mice. *J. Immunol.* 171:3110–18
158. Chakravarty SD, Zhu G, Tsai MC, Mohan VP, Marino S, et al. 2008. Tumor necrosis factor blockade in chronic murine tuberculosis enhances granulomatous inflammation and disorganizes granulomas in the lungs. *Infect. Immun.* 76:916–26

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159. Mohan VP, Scanga CA, Yu K, Scott HM, Tanaka KE, et al. 2001. Effects of tumor necrosis factor α on host immune response in chronic persistent tuberculosis: possible role for limiting pathology. *Infect. Immun.* 69:1847–55
160. Harris J, Keane J. 2010. How tumour necrosis factor blockers interfere with tuberculosis immunity. *Clin. Exp. Immunol.* 161:1–9
161. Vassalli P. 1992. The pathophysiology of tumor necrosis factors. *Annu. Rev. Immunol.* 10:411–52
162. Pasparakis M, Alexopoulou L, Douni E, Kollias G. 1996. Tumour necrosis factors in immune regulation: everything that's interesting is. . .new! *Cytokine Growth Factor Rev.* 7:223–29
163. Roach DR, Briscoe H, Saunders B, France MP, Riminton S, Britton WJ. 2001. Secreted lymphotoxin-α is essential for the control of an intracellular bacterial infection. *J. Exp. Med.* 193:239–46
164. Iliopoulos A, Psathakis K, Aslanidis S, Skagias L, Sfikakis PP. 2006. Tuberculosis and granuloma formation in patients receiving anti-TNF therapy. *Int. J. Tuberc. Lung Dis.* 10:588–90
165. Lin PL, Myers A, Smith L, Bigbee C, Bigbee M, et al. 2010. Tumor necrosis factor neutralization results in disseminated disease in acute and latent *Mycobacterium tuberculosis* infection with normal granuloma structure in a cynomolgus macaque model. *Arthritis Rheum.* 62:340–50
166. Clay H, Volkman HE, Ramakrishnan L. 2008. Tumor necrosis factor signaling mediates resistance to mycobacteria by inhibiting bacterial growth and macrophage death. *Immunity* 29:283–94
167. Miller EA, Ernst JD. 2008. Illuminating the black box of TNF action in tuberculous granulomas. *Immunity* 29:175–77
168. Herb F, Thye T, Niemann S, Browne EN, Chinbuah MA, et al. 2008. *ALOX5* variants associated with susceptibility to human pulmonary tuberculosis. *Hum. Mol. Genet.* 17:1052–60
169. Salgame P. 2011. MMPs in tuberculosis: granuloma creators and tissue destroyers. *J. Clin. Investig.* 121:1686–88
170. Elkington P, Shiomi T, Breen R, Nuttall RK, Ugarte-Gil CA, et al. 2011. MMP-1 drives immunopathology in human tuberculosis and transgenic mice. *J. Clin. Investig.* 121:1827–33
171. Elkington PT, Nuttall RK, Boyle JJ, O'Kane CM, Horncastle DE, et al. 2005. *Mycobacterium tuberculosis*, but not vaccine BCG, specifically upregulates matrix metalloproteinase-1. *Am. J. Respir. Crit. Care Med.* 172:1596–604
172. Elkington PT, Ugarte-Gil CA, Friedland JS. 2011. Matrix metalloproteinases in tuberculosis. *Eur. Respir. J.* 38:456–64
173. Price NM, Farrar J, Tran TT, Nguyen TH, Tran TH, Friedland JS. 2001. Identification of a matrix-degrading phenotype in human tuberculosis in vitro and in vivo. *J. Immunol.* 166:4223–30
174. Volkman HE, Pozos TC, Zheng J, Davis JM, Rawls JF, Ramakrishnan L. 2010. Tuberculous granuloma induction via interaction of a bacterial secreted protein with host epithelium. *Science* 327:466–69
175. Walker NF, Clark SO, Oni T, Andreu N, Tezera L, et al. 2012. Doxycycline and HIV infection suppress tuberculosis-induced matrix metalloproteinases. *Am. J. Respir. Crit. Care Med.* 185:989–97
176. Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, et al. 2011. High-dose vitamin D_3 during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. *Lancet* 377:242–50
177. Nursyam EW, Amin Z, Rumende CM. 2006. The effect of vitamin D as supplementary treatment in patients with moderately advanced pulmonary tuberculous lesion. *Acta Med. Indones.* 38:3–5
178. Martineau AR, Nhamoyebonde S, Oni T, Rangaka MX, Marais S, et al. 2011. Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa. *Proc. Natl. Acad. Sci. USA* 108:19013–17
179. Raghavan S, Alagarasu K, Selvaraj P. 2012. Immunogenetics of HIV and HIV associated tuberculosis. *Tuberculosis* 92:18–30
180. Rook GA, Steele J, Fraher L, Barker S, Karmali R, et al. 1986. Vitamin D_3, gamma interferon, and control of proliferation of *Mycobacterium tuberculosis* by human monocytes. *Immunology* 57:159–63
181. Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, et al. 2009. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. *Cell Host Microbe* 6:231–43
182. Berrington WR, Hawn TR. 2007. *Mycobacterium tuberculosis*, macrophages, and the innate immune response: does common variation matter? *Immunol. Rev.* 219:167–86

Annu. Rev. Immunol. 2013.31:475-527. Downloaded from www.annualreviews.org
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183. Reiling N, Ehlers S, Holscher C. 2008. MyDths and un-TOLLed truths: sensor, instructive and effector immunity to tuberculosis. *Immunol. Lett.* 116:15–23
184. Schafer G, Jacobs M, Wilkinson RJ, Brown GD. 2009. Non-opsonic recognition of *Mycobacterium tuberculosis* by phagocytes. *J. Innate Immun.* 1:231–43
185. Tanne A, Neyrolles O. 2011. C-type lectins in immunity to *Mycobacterium tuberculosis*. *Front. Biosci.* 3:1147–64
186. Divangahi M, Mostowy S, Coulombe F, Kozak R, Guillot L, et al. 2008. NOD2-deficient mice have impaired resistance to *Mycobacterium tuberculosis* infection through defective innate and adaptive immunity. *J. Immunol.* 181:7157–65
187. Tanne A, Ma B, Boudou F, Tailleux L, Botella H, et al. 2009. A murine DC-SIGN homologue contributes to early host defense against *Mycobacterium tuberculosis*. *J. Exp. Med.* 206:2205–20
188. Caws M, Thwaites G, Dunstan S, Hawn TR, Lan NT, et al. 2008. The influence of host and bacterial genotype on the development of disseminated disease with *Mycobacterium tuberculosis*. *PLoS Pathog.* 4:e1000034
189. Fremond CM, Yeremeev V, Nicolle DM, Jacobs M, Quesniaux VF, Ryffel B. 2004. Fatal *Mycobacterium tuberculosis* infection despite adaptive immune response in the absence of MyD88. *J. Clin. Investig.* 114:1790–99
190. Scanga CA, Bafica A, Feng CG, Cheever AW, Hieny S, Sher A. 2004. MyD88-deficient mice display a profound loss in resistance to *Mycobacterium tuberculosis* associated with partially impaired Th1 cytokine and nitric oxide synthase 2 expression. *Infect. Immun.* 72:2400–4
191. Sugawara I, Yamada H, Mizuno S, Takeda K, Akira S. 2003. Mycobacterial infection in MyD88-deficient mice. *Microbiol. Immunol.* 47:841–47
192. Dorhoi A, Desel C, Yeremeev V, Pradl L, Brinkmann V, et al. 2010. The adaptor molecule CARD9 is essential for tuberculosis control. *J. Exp. Med.* 207:777–92
193. Juffermans NP, Florquin S, Camoglio L, Verbon A, Kolk AH, et al. 2000. Interleukin-1 signaling is essential for host defense during murine pulmonary tuberculosis. *J. Infect. Dis.* 182:902–8
194. Sugawara I, Yamada H, Hua S, Mizuno S. 2001. Role of interleukin (IL)-1 type 1 receptor in mycobacterial infection. *Microbiol. Immunol.* 45:743–50
195. Yamada H, Mizumo S, Horai R, Iwakura Y, Sugawara I. 2000. Protective role of interleukin-1 in mycobacterial infection in IL-1 α/β double-knockout mice. *Lab. Investig.* 80:759–67
196. Kleinnijenhuis J, Joosten LA, van de Veerdonk FL, Savage N, van Crevel R, et al. 2009. Transcriptional and inflammasome-mediated pathways for the induction of IL-1β production by *Mycobacterium tuberculosis*. *Eur. J. Immunol.* 39:1914–22
197. Fremond CM, Togbe D, Doz E, Rose S, Vasseur V, et al. 2007. IL-1 receptor-mediated signal is an essential component of MyD88-dependent innate response to *Mycobacterium tuberculosis* infection. *J. Immunol.* 179:1178–89
198. Mayer-Barber KD, Barber DL, Shenderov K, White SD, Wilson MS, et al. 2010. Caspase-1 independent IL-1β production is critical for host resistance to *Mycobacterium tuberculosis* and does not require TLR signaling in vivo. *J. Immunol.* 184:3326–30
199. De Libero G, Mori L. 2008. How T cells get grip on lipid antigens. *Curr. Opin. Immunol.* 20:96–104
200. Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR. 1995. Natural and synthetic non-peptide antigens recognized by human $\gamma\delta$ T cells. *Nature* 375:155–58
201. Steinman RM. 2012. Decisions about dendritic cells: past, present, and future. *Annu. Rev. Immunol.* 30:1–22
202. Tailleux L, Schwartz O, Herrmann JL, Pivert E, Jackson M, et al. 2003. DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. *J. Exp. Med.* 197:121–27
203. Tian T, Woodworth J, Skold M, Behar SM. 2005. In vivo depletion of $CD11c^+$ cells delays the $CD4^+$ T cell response to *Mycobacterium tuberculosis* and exacerbates the outcome of infection. *J. Immunol.* 175:3268–72
204. Urdahl KB, Shafiani S, Ernst JD. 2011. Initiation and regulation of T-cell responses in tuberculosis. *Mucosal Immunol.* 4:288–93
205. Winslow GM, Cooper A, Reiley W, Chatterjee M, Woodland DL. 2008. Early T-cell responses in tuberculosis immunity. *Immunol. Rev.* 225:284–99

Annu. Rev. Immunol. 2013.31:475-527. Downloaded from www.annualreviews.org
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206. Gallegos AM, Pamer EG, Glickman MS. 2008. Delayed protection by ESAT-6-specific effector CD4+ T cells after airborne *M. tuberculosis* infection. *J. Exp. Med.* 205:2359–68
207. Reiley WW, Calayag MD, Wittmer ST, Huntington JL, Pearl JE, et al. 2008. ESAT-6-specific CD4 T cell responses to aerosol *Mycobacterium tuberculosis* infection are initiated in the mediastinal lymph nodes. *Proc. Natl. Acad. Sci. USA* 105:10961–66
208. Wolf AJ, Desvignes L, Linas B, Banaiee N, Tamura T, et al. 2008. Initiation of the adaptive immune response to *Mycobacterium tuberculosis* depends on antigen production in the local lymph node, not the lungs. *J. Exp. Med.* 205:105–15
209. Jung YJ, Ryan L, LaCourse R, North RJ. 2005. Properties and protective value of the secondary versus primary T helper type 1 response to airborne *Mycobacterium tuberculosis* infection in mice. *J. Exp. Med.* 201:1915–24
210. Kursar M, Koch M, Mittrucker HW, Nouailles G, Bonhagen K, et al. 2007. Cutting edge: regulatory T cells prevent efficient clearance of *Mycobacterium tuberculosis*. *J. Immunol.* 178:2661–65
211. Scott-Browne JP, Shafiani S, Tucker-Heard G, Ishida-Tsubota K, Fontenot JD, et al. 2007. Expansion and function of Foxp3-expressing T regulatory cells during tuberculosis. *J. Exp. Med.* 204:2159–69
212. Redford PS, Murray PJ, O'Garra A. 2011. The role of IL-10 in immune regulation during *M. tuberculosis* infection. *Mucosal Immunol.* 4:261–70
213. Brigl M, Brenner MB. 2004. CD1: antigen presentation and T cell function. *Annu. Rev. Immunol.* 22:817–90
214. De Libero G, Mori L. 2005. Recognition of lipid antigens by T cells. *Nat. Rev. Immunol.* 5:485–96
215. Beckman EM, Porcelli SA, Morita CT, Behar SM, Furlong ST, Brenner MB. 1994. Recognition of a lipid antigen by CD1-restricted $\alpha\beta^+$ T cells. *Nature* 372:691–94
216. Kasmar AG, van Rhijn I, Cheng T-Y, Turner M, Seshadri C, et al. 2011. CD1b tetramers bind $\alpha\beta$ T cell receptors to identify a mycobacterial glycolipid-reactive T cell repertoire in humans. *J. Exp. Med.* 208:1741–47
217. Moody DB, Ulrichs T, Muhlecker W, Young DC, Gurcha SS, et al. 2000. CD1c-mediated T-cell recognition of isoprenoid glycolipids in *Mycobacterium tuberculosis* infection. *Nature* 404:884–88
218. Moody DB, Young DC, Cheng TY, Rosat JP, Roura-Mir C, et al. 2004. T cell activation by lipopeptide antigens. *Science* 303:527–31
219. Felio K, Nguyen H, Dascher CC, Choi H-J, Li S, et al. 2009. CD1-restricted adaptive immune responses to *Mycobacteria* in human group 1 CD1 transgenic mice. *J. Exp. Med.* 206:2497–509
220. Maglione PJ, Chan J. 2009. How B cells shape the immune response against *Mycobacterium tuberculosis*. *Eur. J. Immunol.* 39:676–86
221. Phuah JY, Mattila JT, Lin PL, Flynn JL. 2012. Activated B cells in the granulomas of nonhuman primates infected with *Mycobacterium tuberculosis*. *Am. J. Pathol.* 181:508–14
222. Bosio CM, Gardner D, Elkins KL. 2000. Infection of B cell-deficient mice with CDC 1551, a clinical isolate of *Mycobacterium tuberculosis*: delay in dissemination and development of lung pathology. *J. Immunol.* 164:6417–25
223. Maglione PJ, Xu J, Chan J. 2007. B cells moderate inflammatory progression and enhance bacterial containment upon pulmonary challenge with *Mycobacterium tuberculosis*. *J. Immunol.* 178:7222–34
224. Maglione PJ, Xu J, Casadevall A, Chan J. 2008. Fcγ receptors regulate immune activation and susceptibility during *Mycobacterium tuberculosis* infection. *J. Immunol.* 180:3329–38
225. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. 1993. Disseminated tuberculosis in interferon γ gene-disrupted mice. *J. Exp. Med.* 178:2243–47
226. Khader SA, Partida-Sanchez S, Bell G, Jelley-Gibbs DM, Swain S, et al. 2006. Interleukin 12p40 is required for dendritic cell migration and T cell priming after *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 203:1805–15
227. Feng CG, Jankovic D, Kullberg M, Cheever A, Scanga CA, et al. 2005. Maintenance of pulmonary Th1 effector function in chronic tuberculosis requires persistent IL-12 production. *J. Immunol.* 174:4185–92
228. Cooper AM, Kipnis A, Turner J, Magram 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.* 168:1322–27

Annu. Rev. Immunol. 2013.31:475-527. Downloaded from www.annualreviews.org
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229. Holscher C, Atkinson RA, Arendse B, Brown N, Myburgh E, et al. 2001. A protective and agonistic function of IL-12p40 in mycobacterial infection. *J. Immunol.* 167:6957–66
230. Cooper AM, Adams LB, Dalton DK, Appelberg R, Ehlers S. 2002. IFN-γ and NO in mycobacterial disease: new jobs for old hands. *Trends Microbiol.* 10:221–26
231. Wozniak TM, Ryan AA, Britton WJ. 2006. Interleukin-23 restores immunity to *Mycobacterium tuberculosis* infection in IL-12p40-deficient mice and is not required for the development of IL-17-secreting T cell responses. *J. Immunol.* 177:8684–92
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
233. Casanova JL, Abel L. 2002. Genetic dissection of immunity to mycobacteria: the human model. *Annu. Rev. Immunol.* 20:581–620
234. Doffinger R, Patel S, Kumararatne DS. 2005. Human immunodeficiencies that predispose to intracellular bacterial infections. *Curr. Opin. Rheumatol.* 17:440–46
235. Dorman S, Picard C, Lammas D, Heyne K, Vandissel J, et al. 2004. Clinical features of dominant and recessive interferon γ receptor 1 deficiencies. *Lancet* 364:2113–21
236. Jouanguy E, Lamhamedi-Cherradi S, Altare F, Fondaneche MC, Tuerlinckx D, et al. 1997. Partial interferon-γ receptor 1 deficiency in a child with tuberculoid bacillus Calmette-Guérin infection and a sibling with clinical tuberculosis. *J. Clin. Investig.* 100:2658–64
237. Altare F, Ensser A, Breiman A, Reichenbach J, Baghdadi JE, et al. 2001. Interleukin-12 receptor β1 deficiency in a patient with abdominal tuberculosis. *J. Infect. Dis.* 184:231–36
238. Boisson-Dupuis S, El Baghdadi J, Parvaneh N, Bousfiha A, Bustamante J, et al. 2011. IL-12Rβ1 deficiency in two of fifty children with severe tuberculosis from Iran, Morocco, and Turkey. *PLoS ONE* 6:e18524
239. Caragol I, Raspall M, Fieschi C, Feinberg J, Larrosa MN, et al. 2003. Clinical tuberculosis in 2 of 3 siblings with interleukin-12 receptor β1 deficiency. *Clin. Infect. Dis.* 37:302–6
240. Ozbek N, Fieschi C, Yilmaz BT, de Beaucoudrey L, Demirhan B, et al. 2005. Interleukin-12 receptor β1 chain deficiency in a child with disseminated tuberculosis. *Clin. Infect. Dis.* 40:e55–58
241. Bustamante J, Arias AA, Vogt G, Picard C, Galicia LB, et al. 2011. Germline CYBB mutations that selectively affect macrophages in kindreds with X-linked predisposition to tuberculous mycobacterial disease. *Nat. Immunol.* 12:213–21
242. Marquis JF, LaCourse R, Ryan L, North RJ, Gros P. 2009. Disseminated and rapidly fatal tuberculosis in mice bearing a defective allele at IFN regulatory factor 8. *J. Immunol.* 182:3008–15
243. Bogunovic D, Byun M, Durfee LA, Abhyankar A, Sanal O, et al. 2012. Mycobacterial disease and impaired IFN-γ immunity in humans with inherited ISG15 deficiency. *Science* 31:537–39
244. Tabarsi P, Marjani M, Mansouri N, Farnia P, Boisson-Dupuis S, et al. 2011. Lethal tuberculosis in a previously healthy adult with IL-12 receptor deficiency. *J. Clin. Immunol.* 31:537–39
245. Baghdadi JE, Orlova M, Alter A, Ranque B, Chentoufi M, et al. 2006. An autosomal dominant major gene confers predisposition to pulmonary tuberculosis in adults. *J. Exp. Med.* 203:1679–84
246. O'Garra AB, Britton WJ. 2008. Cytokines in tuberculosis. In *Handbook of Tuberculosis: Immunology and Cell Biology*, ed. SHE Kaufmann, WJ Britton, 2:185–225. Weinheim: Wiley-VCH
247. Tsao TC, Huang CC, Chiou WK, Yang PY, Hsieh MJ, Tsao KC. 2002. Levels of interferon-γ and interleukin-2 receptor-α for bronchoalveolar lavage fluid and serum were correlated with clinical grade and treatment of pulmonary tuberculosis. *Int. J. Tuberc. Lung Dis.* 6:720–27
248. Vankayalapati R, Wizel B, Weis SE, Klucar P, Shams H, et al. 2003. Serum cytokine concentrations do not parallel *Mycobacterium tuberculosis*-induced cytokine production in patients with tuberculosis. *Clin. Infect. Dis.* 36:24–28
249. Verbon A, Juffermans N, Van Deventer SJ, Speelman P, Van Deutekom H, Van Der Poll T. 1999. Serum concentrations of cytokines in patients with active tuberculosis (TB) and after treatment. *Clin. Exp. Immunol.* 115:110–13
250. Barnes PF, Lu S, Abrams JS, Wang E, Yamamura M, Modlin RL. 1993. Cytokine production at the site of disease in human tuberculosis. *Infect. Immun.* 61:3482–89
251. Bhattacharyya S, Singla R, Dey AB, Prasad HK. 1999. Dichotomy of cytokine profiles in patients and high-risk healthy subjects exposed to tuberculosis. *Infect. Immun.* 67:5597–603

Annu. Rev. Immunol. 2013.31:475-527. Downloaded from www.annualreviews.org
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252. Hirsch CS, Toossi Z, Vanham G, Johnson JL, Peters P, et al. 1999. Apoptosis and T cell hyporesponsiveness in pulmonary tuberculosis. *J. Infect. Dis.* 179:945–53
253. Smith SM, Klein MR, Malin AS, Sillah J, McAdam KP, Dockrell HM. 2002. Decreased IFN-γ and increased IL-4 production by human $CD8^+$ T cells in response to *Mycobacterium tuberculosis* in tuberculosis patients. *Tuberculosis* 82:7–13
254. Song CH, Kim HJ, Park JK, Lim JH, Kim UO, et al. 2000. Depressed interleukin-12 (IL-12), but not IL-18, production in response to a 30- or 32-kilodalton mycobacterial antigen in patients with active pulmonary tuberculosis. *Infect. Immun.* 68:4477–84
255. Torres M, Herrera T, Villareal H, Rich EA, Sada E. 1998. Cytokine profiles for peripheral blood lymphocytes from patients with active pulmonary tuberculosis and healthy household contacts in response to the 30-kilodalton antigen of *Mycobacterium tuberculosis*. *Infect. Immun.* 66:176–80
256. Vekemans J, Lienhardt C, Sillah JS, Wheeler JG, Lahai GP, et al. 2001. Tuberculosis contacts but not patients have higher γ interferon responses to ESAT-6 than do community controls in The Gambia. *Infect. Immun.* 69:6554–57
257. Pathan AA, Wilkinson KA, Klenerman P, McShane H, Davidson RN, et al. 2001. Direct ex vivo analysis of antigen-specific IFN-γ-secreting CD4 T cells in *Mycobacterium tuberculosis*-infected individuals: associations with clinical disease state and effect of treatment. *J. Immunol.* 167:5217–25
258. Sodhi A, Gong J, Silva C, Qian D, Barnes PF. 1997. Clinical correlates of interferon γ production in patients with tuberculosis. *Clin. Infect. Dis.* 25:617–20
259. Hirsch CS, Toossi Z, Othieno C, Johnson JL, Schwander SK, et al. 1999. Depressed T-cell interferon-γ responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. *J. Infect. Dis.* 180:2069–73
260. Cardoso FL, Antas PR, Milagres AS, Geluk A, Franken KL, et al. 2002. T-cell responses to the *Mycobacterium tuberculosis*-specific antigen ESAT-6 in Brazilian tuberculosis patients. *Infect. Immun.* 70:6707–14
261. Dlugovitzky D, Bay ML, Rateni L, Fiorenza G, Vietti L, et al. 2000. Influence of disease severity on nitrite and cytokine production by peripheral blood mononuclear cells (PBMC) from patients with pulmonary tuberculosis (TB). *Clin. Exp. Immunol.* 122:343–49
262. Surcel HM, Troye-Blomberg M, Paulie S, Andersson G, Moreno C, et al. 1994. Th1/Th2 profiles in tuberculosis, based on the proliferation and cytokine response of blood lymphocytes to mycobacterial antigens. *Immunology* 81:171–76
263. Winkler S, Necek M, Winkler H, Adegnika AA, Perkmann T, et al. 2005. Increased specific T cell cytokine responses in patients with active pulmonary tuberculosis from Central Africa. *Microbes Infect.* 7:1161–69
264. Vordermeier HM, Chambers MA, Cockle PJ, Whelan AO, Simmons J, Hewinson RG. 2002. Correlation of ESAT-6-specific γ interferon production with pathology in cattle following *Mycobacterium bovis* BCG vaccination against experimental bovine tuberculosis. *Infect. Immun.* 70:3026–32
265. Zea AH, Ochoa MT, Ghosh P, Longo DL, Alvord WG, et al. 1998. Changes in expression of signal transduction proteins in T lymphocytes of patients with leprosy. *Infect. Immun.* 66:499–504
266. Baliko Z, Szereday L, Szekeres-Bartho J. 1998. Th2 biased immune response in cases with active *Mycobacterium tuberculosis* infection and tuberculin anergy. *FEMS Immunol. Med. Microbiol.* 22:199–204
267. Boussiotis VA, Tsai EY, Yunis EJ, Thim S, Delgado JC, et al. 2000. IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. *J. Clin. Investig.* 105:1317–25
268. Nash DR, Douglass JE. 1980. Anergy in active pulmonary tuberculosis. A comparison between positive and negative reactors and an evaluation of 5 TU and 250 TU skin test doses. *Chest* 77:32–37
269. Zea AH, Culotta KS, Ali J, Mason C, Park HJ, et al. 2006. Decreased expression of CD3ζ and nuclear transcription factor κB in patients with pulmonary tuberculosis: potential mechanisms and reversibility with treatment. *J. Infect. Dis.* 194:1385–93
270. Boehm U, Klamp T, Groot M, Howard JC. 1997. Cellular responses to interferon-γ. *Annu. Rev. Immunol.* 15:749–95
271. MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, Nathan CF. 1997. Identification of nitric oxide synthase as a protective locus against tuberculosis. *Proc. Natl. Acad. Sci. USA*. 94:5243–48

Annu. Rev. Immunol. 2013.31:475-527. Downloaded from www.annualreviews.org
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272. Scanga CA, Mohan VP, Tanaka K, Alland D, Flynn JL, Chan J. 2001. The inducible nitric oxide synthase locus confers protection against aerogenic challenge of both clinical and laboratory strains of *Mycobacterium tuberculosis* in mice. *Infect. Immun.* 69:7711–17
273. Choi HS, Rai PR, Chu HW, Cool C, Chan ED. 2002. Analysis of nitric oxide synthase and nitrotyrosine expression in human pulmonary tuberculosis. *Am. J. Respir. Crit. Care Med.* 166:178–86
274. Nathan C, Shiloh MU. 2000. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc. Natl. Acad. Sci. USA* 97:8841–48
275. MacMicking JD, Taylor GA, McKinney JD. 2003. Immune control of tuberculosis by IFN-γ-inducible LRG-47. *Science* 302:654–59
276. Feng CG, Zheng L, Jankovic D, Bafica A, Cannons JL, et al. 2008. The immunity-related GTPase Irgm1 promotes the expansion of activated $CD4^+$ T cell populations by preventing interferon-γ-induced cell death. *Nat. Immunol.* 9:1279–87
277. Russell DG, VanderVen BC, Lee W, Abramovitch RB, Kim MJ, et al. 2010. *Mycobacterium tuberculosis* wears what it eats. *Cell Host Microbe* 8:68–76
278. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. 2008. PD-1 and its ligands in tolerance and immunity. *Annu. Rev. Immunol.* 26:677–704
279. Jurado JO, Alvarez IB, Pasquinelli V, Martinez GJ, Quiroga MF, et al. 2008. Programmed death (PD)-1:PD-ligand 1/PD-ligand 2 pathway inhibits T cell effector functions during human tuberculosis. *J. Immunol.* 181:116–25
280. McNab FW, Berry MP, Graham CM, Bloch SA, Oni T, et al. 2011. Programmed death ligand 1 is overexpressed by neutrophils in the blood of patients with active tuberculosis. *Eur. J. Immunol.* 41:1941–47
281. Barber DL, Mayer-Barber KD, Antonelli LR, Wilson MS, White S, et al. 2010. Th1-driven immune reconstitution disease in *Mycobacterium avium*-infected mice. *Blood* 116:3485–93
282. Lázár-Molnár E, Chen B, Sweeney KA, Wang EJ, Liu W, et al. 2010. Programmed death-1 (PD-1)-deficient mice are extraordinarily sensitive to tuberculosis. *Proc. Natl. Acad. Sci. USA* 107:13402–7
283. Cruz A, Khader SA, Torrado E, Fraga A, Pearl JE, et al. 2006. Cutting edge: IFN-γ regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. *J. Immunol.* 177:1416–20
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
285. Umemura M, Yahagi A, Hamada S, Begum MD, Watanabe H, et al. 2007. IL-17-mediated regulation of innate and acquired immune response against pulmonary *Mycobacterium bovis* bacille Calmette-Guerin infection. *J. Immunol.* 178:3786–96
286. Okamoto Yoshida Y, Umemura M, Yahagi A, O'Brien RL, Ikuta K, et al. 2010. Essential role of IL-17A in the formation of a mycobacterial infection-induced granuloma in the lung. *J. Immunol.* 184:4414–22
287. Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, et al. 2007. IL-23 and IL-17 in the establishment of protective pulmonary $CD4^+$ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat. Immunol.* 8:369–77
288. Gopal R, Lin Y, Obermajer N, Slight S, Nuthalapati N, et al. 2012. IL-23-dependent IL-17 drives Th1-cell responses following *Mycobacterium bovis* BCG vaccination. *Eur. J. Immunol.* 42:364–73
289. Gallegos AM, van Heijst JW, Samstein M, Su X, Pamer EG, Glickman MS. 2011. A gamma interferon independent mechanism of CD4 T cell mediated control of *M. tuberculosis* infection in vivo. *PLoS Pathog.* 7:e1002052
290. Saraiva M, O'Garra A. 2010. The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10:170–81
291. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. 1991. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* 147:3815–22
292. Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, et al. 1991. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J. Immunol.* 146:3444–51
293. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19:683–765
294. Bogdan C, Vodovotz Y, Nathan C. 1991. Macrophage deactivation by interleukin 10. *J. Exp. Med.* 174:1549–55

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295. Almeida AS, Lago PM, Boechat N, Huard RC, Lazzarini LC, et al. 2009. Tuberculosis is associated with a down-modulatory lung immune response that impairs Th1-type immunity. *J. Immunol.* 183:718–31
296. Huard RC, Chitale S, Leung M, Lazzarini LC, Zhu H, et al. 2003. The *Mycobacterium tuberculosis* complex-restricted gene *cfp32* encodes an expressed protein that is detectable in tuberculosis patients and is positively correlated with pulmonary interleukin-10. *Infect. Immun.* 71:6871–83
297. Bonecini-Almeida MG, Ho JL, Boechat N, Huard RC, Chitale S, et al. 2004. Down-modulation of lung immune responses by interleukin-10 and transforming growth factor β (TGF-β) and analysis of TGF-β receptors I and II in active tuberculosis. *Infect. Immun.* 72:2628–34
298. Rojas RE, Balaji KN, Subramanian A, Boom WH. 1999. Regulation of human $CD4^+$ αβ T-cell-receptor-positive (TCR^+) and γδ TCR^+ T-cell responses to *Mycobacterium tuberculosis* by interleukin-10 and transforming growth factor β. *Infect. Immun.* 67:6461–72
299. Gong JH, Zhang M, Modlin RL, Linsley PS, Iyer D, et al. 1996. Interleukin-10 downregulates *Mycobacterium tuberculosis*-induced Th1 responses and CTLA-4 expression. *Infect. Immun.* 64:913–18
300. Zhang M, Gong J, Iyer DV, Jones BE, Modlin RL, Barnes PF. 1994. T cell cytokine responses in persons with tuberculosis and human immunodeficiency virus infection. *J. Clin. Investig.* 94:2435–42
301. Gerosa F, Nisii C, Righetti S, Micciolo R, Marchesini M, et al. 1999. $CD4^+$ T cell clones producing both interferon-γ and interleukin-10 predominate in bronchoalveolar lavages of active pulmonary tuberculosis patients. *Clin. Immunol.* 92:224–34
302. Awomoyi AA, Marchant A, Howson JM, McAdam KP, Blackwell JM, Newport MJ. 2002. Interleukin-10, polymorphism in SLC11A1 (formerly NRAMP1), and susceptibility to tuberculosis. *J. Infect. Dis.* 186:1808–14
303. Pacheco AG, Cardoso CC, Moraes MO. 2008. *IFNG* +874T/A, *IL10* -1082G/A and *TNF* -308G/A polymorphisms in association with tuberculosis susceptibility: a meta-analysis study. *Hum. Genet.* 123:477–84
304. Zhang J, Chen Y, Nie XB, Wu WH, Zhang H, et al. 2011. Interleukin-10 polymorphisms and tuberculosis susceptibility: a meta-analysis. *Int. J. Tuberc. Lung Dis.* 15:594–601
305. Jung YJ, Ryan L, LaCourse R, North RJ. 2003. Increased interleukin-10 expression is not responsible for failure of T helper 1 immunity to resolve airborne *Mycobacterium tuberculosis* infection in mice. *Immunology* 109:295–99
306. North RJ. 1998. Mice incapable of making IL-4 or IL-10 display normal resistance to infection with *Mycobacterium tuberculosis*. *Clin. Exp. Immunol.* 113:55–58
307. Beamer GL, Flaherty DK, Assogba BD, Stromberg P, Gonzalez-Juarrero M, et al. 2008. Interleukin-10 promotes *Mycobacterium tuberculosis* disease progression in CBA/J mice. *J. Immunol.* 181:5545–50
308. Turner J, Gonzalez-Juarrero M, Ellis DL, Basaraba RJ, Kipnis A, et al. 2002. In vivo IL-10 production reactivates chronic pulmonary tuberculosis in C57BL/6 mice. *J. Immunol.* 169:6343–51
309. Higgins DM, Sanchez-Campillo J, Rosas-Taraco AG, Lee EJ, Orme IM, Gonzalez-Juarrero M. 2009. Lack of IL-10 alters inflammatory and immune responses during pulmonary *Mycobacterium tuberculosis* infection. *Tuberculosis* 89:149–57
310. Roach DR, Martin E, Bean AG, Rennick DM, Briscoe H, Britton WJ. 2001. Endogenous inhibition of antimycobacterial immunity by IL-10 varies between mycobacterial species. *Scand. J. Immunol.* 54:163–70
311. Demangel C, Bertolino P, Britton WJ. 2002. Autocrine IL-10 impairs dendritic cell (DC)-derived immune responses to mycobacterial infection by suppressing DC trafficking to draining lymph nodes and local IL-12 production. *Eur. J. Immunol.* 32:994–1002
312. O'Leary S, O'Sullivan MP, Keane J. 2011. IL-10 blocks phagosome maturation in *Mycobacterium tuberculosis*-infected human macrophages. *Am. J. Respir. Cell Mol. Biol.* 45:172–80
313. Shaw TC, Thomas LH, Friedland JS. 2000. Regulation of IL-10 secretion after phagocytosis of *Mycobacterium tuberculosis* by human monocytic cells. *Cytokine* 12:483–86
314. Schreiber T, Ehlers S, Heitmann L, Rausch A, Mages J, et al. 2009. Autocrine IL-10 induces hallmarks of alternative activation in macrophages and suppresses antituberculosis effector mechanisms without compromising T cell immunity. *J. Immunol.* 183:1301–12
315. Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, et al. 2007. Regulatory T cells expressing interleukin 10 develop from $Foxp3^+$ and $Foxp3^-$ precursor cells in the absence of interleukin 10. *Nat. Immunol.* 8:931–41

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316. Ordway D, Henao-Tamayo M, Harton M, Palanisamy G, Troudt J, et al. 2007. The hypervirulent *Mycobacterium tuberculosis* strain HN878 induces a potent TH1 response followed by rapid down-regulation. *J. Immunol.* 179:522–31
317. Newton SM, Smith RJ, Wilkinson KA, Nicol MP, Garton NJ, et al. 2006. A deletion defining a common Asian lineage of *Mycobacterium tuberculosis* associates with immune subversion. *Proc. Natl. Acad. Sci. USA* 103:15594–98
318. Pitt JM, Stavropoulos E, Redford PS, Beebe AM, Bancroft GJ, et al. 2012. Blockade of IL-10 signaling during bacillus Calmette-Guérin vaccination enhances and sustains Th1, Th17, and innate lymphoid IFN-γ and IL-17 responses and increases protection to *Mycobacterium tuberculosis* infection. *J. Immunol.* 189:4079–87
319. Elias D, Britton S, Aseffa A, Engers H, Akuffo H. 2008. Poor immunogenicity of BCG in helminth infected population is associated with increased in vitro TGF-β production. *Vaccine* 26:3897–902
320. Resende Co T, Hirsch CS, Toossi Z, Dietze R, Ribeiro-Rodrigues R. 2007. Intestinal helminth co-infection has a negative impact on both anti-*Mycobacterium tuberculosis* immunity and clinical response to tuberculosis therapy. *Clin. Exp. Immunol.* 147:45–52
321. Babu S, Bhat SQ, Kumar NP, Jayantasri S, Rukmani S, et al. 2009. Human type 1 and 17 responses in latent tuberculosis are modulated by coincident filarial infection through cytotoxic T lymphocyte antigen-4 and programmed death-1. *J. Infect. Dis.* 200:288–98
322. Potian JA, Rafi W, Bhatt K, McBride A, Gause WC, Salgame P. 2011. Preexisting helminth infection induces inhibition of innate pulmonary anti-tuberculosis defense by engaging the IL-4 receptor pathway. *J. Exp. Med.* 208:1863–74
323. Trinchieri G. 2010. Type I interferon: friend or foe? *J. Exp. Med.* 207:2053–63
324. Cooper AM, Pearl JE, Brooks JV, Ehlers S, Orme IM. 2000. Expression of the nitric oxide synthase 2 gene is not essential for early control of *Mycobacterium tuberculosis* in the murine lung. *Infect. Immun.* 68:6879–82
325. Stanley SA, Johndrow JE, Manzanillo P, Cox JS. 2007. The type I IFN response to infection with *Mycobacterium tuberculosis* requires ESX-1-mediated secretion and contributes to pathogenesis. *J. Immunol.* 178:3143–52
326. Antonelli LR, Gigliotti Rothfuchs A, Gonçalves R, Roffe E, Cheever AW, et al. 2010. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. *J. Clin. Investig.* 120:1674–82
327. Kaiser F, Cook D, Papoutsopoulou S, Rajsbaum R, Wu X, et al. 2009. TPL-2 negatively regulates interferon-β production in macrophages and myeloid dendritic cells. *J. Exp. Med.* 206:1863–71
328. Pandey AK, Yang Y, Jiang Z, Fortune SM, Coulombe F, et al. 2009. NOD2, RIP2 and IRF5 play a critical role in the type I interferon response to *Mycobacterium tuberculosis*. *PLoS Pathog.* 5:e1000500
329. Novikov A, Cardone M, Thompson R, Shenderov K, Kirschman KD, et al. 2011. *Mycobacterium tuberculosis* triggers host type I IFN signaling to regulate IL-1β production in human macrophages. *J. Immunol.* 187:2540–47
330. Mayer-Barber KD, Andrade BB, Barber DL, Hieny S, Feng CG, et al. 2011. Innate and adaptive interferons suppress IL-1α and IL-1β production by distinct pulmonary myeloid subsets during *Mycobacterium tuberculosis* infection. *Immunity* 35:1023–34
331. Mariotti S, Teloni R, Iona E, Fattorini L, Romagnoli G, et al. 2004. *Mycobacterium tuberculosis* diverts α interferon-induced monocyte differentiation from dendritic cells into immunoprivileged macrophage-like host cells. *Infect. Immun.* 72:4385–92
332. Reed MB, Domenech P, Manca C, Su H, Barczak AK, et al. 2004. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. *Nature* 431:84–87
333. Portevin D, Gagneux S, Comas I, Young D. 2011. Human macrophage responses to clinical isolates from the *Mycobacterium tuberculosis* complex discriminate between ancient and modern lineages. *PLoS Pathog.* 7:e1001307
334. Filliol I, Motiwala AS, Cavatore M, Qi W, Hazbon MH, et al. 2006. Global phylogeny of *Mycobacterium tuberculosis* based on single nucleotide polymorphism (SNP) analysis: insights into tuberculosis evolution, phylogenetic accuracy of other DNA fingerprinting systems, and recommendations for a minimal standard SNP set. *J. Bacteriol.* 188:759–72

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335. Tsenova L, Ellison E, Harbacheuski R, Moreira AL, Kurepina N, et al. 2005. Virulence of selected *Mycobacterium tuberculosis* clinical isolates in the rabbit model of meningitis is dependent on phenolic glycolipid produced by the bacilli. *J. Infect. Dis.* 192:98–106
336. Sinsimer D, Huet G, Manca C, Tsenova L, Koo MS, et al. 2008. The phenolic glycolipid of *Mycobacterium tuberculosis* differentially modulates the early host cytokine response but does not in itself confer hypervirulence. *Infect. Immun.* 76:3027–36
337. Koo MS, Subbian S, Kaplan G. 2012. Strain specific transcriptional response in *Mycobacterium tuberculosis* infected macrophages. *Cell Commun. Signal.* 10:2
338. Schutz C, Meintjes G, Almajid F, Wilkinson RJ, Pozniak A. 2010. Clinical management of tuberculosis and HIV-1 co-infection. *Eur. Respir. J.* 36:1460–81
339. Rangaka MX, Gideon HP, Wilkinson KA, Pai M, Mwansa-Kambafwile J, et al. 2012. Interferon release does not add discriminatory value to smear-negative HIV-tuberculosis algorithms. *Eur. Respir. J.* 39:163–71
340. Johnson JL, Nyole S, Okwera A, Whalen CC, Nsubuga P, et al. 1998. Instability of tuberculin and candida skin test reactivity in HIV-infected Ugandans. *Am. J. Respir. Crit. Care Med.* 158:1790–96
341. Rangaka MX, Wilkinson KA, Seldon R, Van Cutsem G, Meintjes GA, et al. 2007. Effect of HIV-1 infection on T-cell-based and skin test detection of tuberculosis infection. *Am. J. Respir. Crit. Care Med.* 175:514–20
342. Meintjes G, Lawn SD, Scano F, Maartens G, French MA, et al. 2008. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. *Lancet Infect. Dis.* 8:516–23
343. Mukadi Y, Perriëns JH, St Louis ME, Brown C, Prignot J, et al. 1993. Spectrum of immunodeficiency in HIV-1-infected patients with pulmonary tuberculosis in Zaire. *Lancet* 342:143–46
344. Sonnenberg P, Glynn JR, Fielding K, Murray J, Godfrey-Faussett P, Shearer S. 2005. How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. *J. Infect. Dis.* 191:150–58
345. Kalsdorf B, Scriba TJ, Wood K, Day CL, Dheda K, et al. 2009. HIV-1 infection impairs the bronchoalveolar T-cell response to mycobacteria. *Am. J. Respir. Crit. Care Med.* 180:1262–70
346. Matthews K, Ntsekhe M, Syed F, Scriba T, Russell J, et al. 2012. HIV-1 infection alters $CD4^+$ memory T-cell phenotype at the site of disease in extrapulmonary tuberculosis. *Eur. J. Immunol.* 42:147–57
347. Hill AR, Premkumar S, Brustein S, Vaidya K, Powell S, et al. 1991. Disseminated tuberculosis in the acquired immunodeficiency syndrome era. *Am. Rev. Respir. Dis.* 144:1164–70
348. Mattila JT, Diedrich CR, Lin PL, Phuah J, Flynn JL. 2011. Simian immunodeficiency virus-induced changes in T cell cytokine responses in cynomolgus macaques with latent *Mycobacterium tuberculosis* infection are associated with timing of reactivation. *J. Immunol.* 186:3527–37
349. Pathak S, Wentzel-Larsen T, Asjo B. 2010. Effects of in vitro HIV-1 infection on mycobacterial growth in peripheral blood monocyte-derived macrophages. *Infect. Immun.* 78:4022–32
350. Meylan PR, Munis JR, Richman DD, Kornbluth RS. 1992. Concurrent human immunodeficiency virus and mycobacterial infection of macrophages in vitro does not reveal any reciprocal effect. *J. Infect. Dis.* 165:80–86
351. Reuter MA, Pecora ND, Harding CV, Canaday DH, McDonald D. 2010. *Mycobacterium tuberculosis* promotes HIV trans-infection and suppresses major histocompatibility complex class II antigen processing by dendritic cells. *J. Virol.* 84:8549–60
352. Campbell GR, Spector SA. 2012. Vitamin D inhibits human immunodeficiency virus type 1 and *Mycobacterium tuberculosis* infection in macrophages through the induction of autophagy. *PLoS Pathog.* 8:e1002689
353. Hoshino Y, Tse DB, Rochford G, Prabhakar S, Hoshino S, et al. 2004. *Mycobacterium tuberculosis*-induced CXCR4 and chemokine expression leads to preferential X4 HIV-1 replication in human macrophages. *J. Immunol.* 172:6251–58
354. Toossi Z, Johnson JL, Kanost RA, Wu M, Luzze H, et al. 2001. Increased replication of HIV-1 at sites of *Mycobacterium tuberculosis* infection: potential mechanisms of viral activation. *J. Acquir. Immune Defic. Syndr.* 28:1–8

Annu. Rev. Immunol. 2013.31:475-527. Downloaded from www.annualreviews.org
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355. Hoshino Y, Hoshino S, Gold JA, Raju B, Prabhakar S, et al. 2007. Mechanisms of polymorphonuclear neutrophil-mediated induction of HIV-1 replication in macrophages during pulmonary tuberculosis. *J. Infect. Dis.* 195:1303–10
356. Hoshino Y, Nakata K, Hoshino S, Honda Y, Tse DB, et al. 2002. Maximal HIV-1 replication in alveolar macrophages during tuberculosis requires both lymphocyte contact and cytokines. *J. Exp. Med.* 195:495–505
357. Toossi Z, Wu M, Hirsch CS, Mayanja-Kizza H, Baseke J, et al. 2012. Activation of P-TEFb at sites of dual HIV/TB infection, and inhibition of MTB-induced HIV transcriptional activation by the inhibitor of CDK9, Indirubin-3′-monoxime. *AIDS Res. Hum. Retrovir.* 28:182–87
358. Hengel RL, Allende MC, Dewar RL, Metcalf JA, Mican JM, Lane HC. 2002. Increasing $CD4^+$ T cells specific for tuberculosis correlate with improved clinical immunity after highly active antiretroviral therapy. *AIDS Res. Hum. Retrovir.* 18:969–75
359. Sutherland JS, Young JM, Peterson KL, Sanneh B, Whittle HC, et al. 2010. Polyfunctional $CD4^+$ and $CD8^+$ T cell responses to tuberculosis antigens in HIV-1-infected patients before and after antiretroviral treatment. *J. Immunol.* 184:6537–44
360. Wilkinson KA, Seldon R, Meintjes G, Rangaka MX, Hanekom WA, et al. 2009. Dissection of regenerating T-cell responses against tuberculosis in HIV-infected adults sensitized by *Mycobacterium tuberculosis*. *Am. J. Respir. Crit. Care Med.* 180:674–83
361. Meintjes G, Rabie H, Wilkinson RJ, Cotton MF. 2009. Tuberculosis-associated immune reconstitution inflammatory syndrome and unmasking of tuberculosis by antiretroviral therapy. *Clin. Chest Med.* 30:797–810
362. Narita M, Ashkin D, Hollender ES, Pitchenik AE. 1998. Paradoxical worsening of tuberculosis following antiretroviral therapy in patients with AIDS. *Am. J. Respir. Crit. Care Med.* 158:157–61
363. Bourgarit A, Carcelain G, Martinez V, Lascoux C, Delcey V, et al. 2006. Explosion of tuberculin-specific Th1-responses induces immune restoration syndrome in tuberculosis and HIV co-infected patients. *AIDS* 20:F1–7
364. Meintjes G, Skolimowska KH, Wilkinson KA, Matthews K, Tadokera R, et al. 2012. Corticosteroid modulated immune activation in the TB immune reconstitution inflammatory syndrome. *Am. J. Respir. Crit. Care Med.* 186:369–77
365. Meintjes G, Wilkinson KA, Rangaka MX, Skolimowska K, van Veen K, et al. 2008. Type 1 helper T cells and FoxP3-positive T cells in HIV-tuberculosis-associated immune reconstitution inflammatory syndrome. *Am. J. Respir. Crit. Care Med.* 178:1083–89
366. Seddiki N, Sasson SC, Santner-Nanan B, Munier M, van Bockel D, et al. 2009. Proliferation of weakly suppressive regulatory $CD4^+$ T cells is associated with over-active $CD4^+$ T-cell responses in HIV-positive patients with mycobacterial immune restoration disease. *Eur. J. Immunol.* 39:391–403
367. Barber DL, Andrade BB, Sereti I, Sher A. 2012. Immune reconstitution inflammatory syndrome: the trouble with immunity when you had none. *Nat. Rev. Microbiol.* 10:150–56
368. Lawn SD, Wainwright H, Orrell C. 2009. Fatal unmasking tuberculosis immune reconstitution disease with bronchiolitis obliterans organizing pneumonia: the role of macrophages. *AIDS* 23:143–45
369. Tadokera R, Meintjes G, Skolimowska KH, Wilkinson KA, Matthews K, et al. 2011. Hypercytokinaemia accompanies HIV-tuberculosis immune reconstitution inflammatory syndrome. *Eur. Respir. J.* 37:1248–59
370. Bourgarit A, Carcelain G, Samri A, Parizot C, Lafaurie M, et al. 2009. Tuberculosis-associated immune restoration syndrome in HIV-1-infected patients involves tuberculin-specific CD4 Th1 cells and KIR-negative $\gamma\delta$ T cells. *J. Immunol.* 183:3915–23
371. Pean P, Nerrienet E, Madec Y, Borand L, Laureillard D, et al. 2012. Natural killer cell degranulation capacity predicts early onset of the immune reconstitution inflammatory syndrome (IRIS) in HIV-infected patients with tuberculosis. *Blood* 119:3315–20
372. Round JL, Mazmanian SK. 2009. The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* 9:313–23
373. Karp CL. 2012. Unstressing intemperate models: how cold stress undermines mouse modeling. *J. Exp. Med.* 209:1069–74

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