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TUBERCULOSIS

TB or Not TB: That Is No Longer the Question

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Tuberculosis (TB) remains a devastating infectious disease and, with the emergence of multidrug-resistant forms, represents a major global threat. Much of our understanding of pathogenic and immunologic mechanisms in TB has derived from studies in experimental animals. However, it is becoming increasingly clear in TB as well as in other inflammatory diseases that there are substantial differences in immunological responses of humans not found or predicted by animal studies. Thus, it is critically important to understand mechanisms of pathogenesis and immunological protection in humans. In this review, we will address the key immunological question: What are the necessary and sufficient immune responses required for protection against TB infection and disease in people—specifically protection against infection, protection against the establishment of latency or persistence, and protection against transitioning from latent infection to active disease.

INTRODUCTION

Tuberculosis (TB) is a disease of global proportions that continues to pose a major infectious disease threat. Worldwide, 8.7 million new cases and 1.4 million deaths were reported in 2011 alone (*1*). In the United States, estimates are that 10 million to 15 million people are infected with *Mycobacterium tuberculosis* (*Mtb*) (*2*, *3*). Twenty-two high-burden countries account for more than 80% of the world's TB cases, and TB is the leading attributable cause of death in individuals infected with HIV (*2*, *4*, *5*) and a major cause of death in women of reproductive age worldwide. *Mtb* is almost exclusively a human pathogen and has adapted exquisitely to a life cycle that enables it to persist latently, but is capable directly or through reactivation of causing sufficient lung pathology to assure its aerosol transmission. Thus, the major risk factor for acquiring TB is breathing; as such, everyone is at risk.

The recent emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB in individuals in KwaZulu Natal and more than 70 other countries represents an alarming new threat (*6*, *7*) not only to patients but to health care workers as well. Of the estimated 8.7 million people that acquired TB in 2011, about 300,000 were infected with MDR or XDR TB. However, because of the limited capacity for rapid diagnosis and drug resistance testing in most developing countries, the burden of MDR and XDR TB is almost certainly greater than these reported values. Although *Mtb* causes disease in only 5 to 10% of those infected in a lifetime, in immunodeficient individuals, the risk of disease increases to ~8% a year. Moreover, drug-resistant TB carries a high risk of mortality: XDR TB was found to cause death in as short a time as a mean of 16 days after diagnosis in HIV^+ patients (*6*). Only about 20% of MDR patients are receiving appropriate second-line drug treatment, and in only half of these patients is treatment reported to have been successful (*1*, *7*, *8*). The paucity of effective second-line drugs in MDR and XDR patients, their high cost, and lack of drug availability in high-burden countries underscore the need for greater efforts at prevention. Thus, understanding the human immune mechanisms involved in protection and in pathogenesis has become a major and urgent research issue.

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LEARNING FROM ANIMAL MODELS

There have been considerable advances in our understanding of *Mtb* infection from investigation of experimental animal models (*9*–*13*). For example, the study of knockout mouse strains established the importance of both $CD4^+$ and $CD8^+$ T cells, as well as cytokines, such as interferon-γ (IFN-γ) and tumor necrosis factor–α (TNF-α), in protection (*14*, *15*), and mouse models of TB are critical for drug screening (*16*). Studies in the rabbit model elucidated the process of granuloma formation and allowed for the study of TB meningitis (*17*–*19*). Studies in nonhuman primates revealed that each granuloma is essentially initiated by a single organism, that the inflammatory processes are dynamic in that infected lymph nodes show great but transient patterns of metabolic bursts, and that the frequency of mutations in the *Mtb* genome in rapidly progressive, latent, and relapsing TB is essentially comparable and mostly attributable to oxidative DNA damage (*9*, *10*, *20*). Even studies in zebrafish have been informative, revealing lipid pathways that could contribute to increased protection or pathogenesis (*21*).

Many of the findings in animal models have been corroborated by studies of human TB. For example, individuals with genetic mutations in the IFN-γ pathway (*22*–*24*) and patients with arthritis treated with anti–TNF-α monoclonal antibodies are more susceptible to mycobacterial infection than healthy controls (*25*). The presence of foamy macrophages has been observed among both mouse and human alveolar macrophages (*26*), leading to studies demonstrating the importance of lipid metabolism in regulating host defense in mouse (*27*) and human disease (*28*, *29*). In addition, B cells have been observed to form germinal-like centers in both a mouse model and human disease (*30*), although it is not clear if B cells contribute to protection or pathogenesis (*31*, *32*). However, even though animal models have been important to the development of new strategies to prevent and treat TB, better ones are needed, for example, humanized mice reconstituted so that they have more human macrophages (*33*). Nonhuman primates are another essential model (*9*); however, some human immune pathways in these animals, such as the vitamin D antimicrobial pathway, remain to be characterized, and the extent to which they faithfully represent human responses is an important research agenda.

LEARNING FROM HUMANS

Despite advances made in animal models, human TB shows important differences from the mouse and other models in clinical presentation,

pathology, and immunologic responses. Clinically, human TB exhibits a diversity of presentations and manifestations, from localized granulomas to disseminated and miliary disease, with a latent or persistent state for most of the individuals infected with *Mtb*. Although the vast majority of humans infected with *Mtb*, as detected by skin test conversion or IFN-γ release assays (IGRAs), control the infection or develop latent infection, mice infected with *Mtb*, whether immunized or not, do not exhibit latent infection and ultimately succumb to the disease. Immunologically, although there are common control pathways in both animal models and human patients, humans also use specific mechanisms that are either not present or not yet characterized in most experimental models. Furthermore, the importance of understanding human immune responses in TB was underscored by the marked results of a recent phase 2b clinical trial of the first new vaccine against TB in over a half century, in which 2800 Bacille Calmette-Guérin (BCG)–vaccinated infants were boosted with a vaccine composed of an attenuated modified vaccinia virus Ankara (MVA) expressing *Mtb* antigen 85A. Although this vaccine had shown some protection in mouse, guinea pigs, and nonhuman primate models of TB (*34*–*37*), and it induced $CD4^+$ T cell responses to *Mtb* antigen 85A in humans, it failed to engender any protection against either infection or disease in the clinical trial (*38*).

Although different forms of TB have long been noted, for example, primary progressive, childhood TB, extrapulmonary, latent, and reactivation disease (*39*), to the clinician the diagnosis and treatment of TB is largely a binary decision—either the patient has TB or not. However, recent thinking based on understanding the underlying pathogenetic mechanisms (*40*) is that TB, like leprosy, is not a static disease but a dynamic one, both clinically (*10*, *13*, *40*) and immunologically, and represents a spectrum of clinical and immunological responses rather than a single disease entity. Hence, it will be increasingly important to understand mechanisms of protection and pathogenesis in humans. This perspective is consistent with current trends in the field, which emphasize the limitations of predicting human inflammatory responses from animal models (*41*, *42*). We focus here on what is known about human immune responses in TB, and where there appear to be significant differences from the study of animal models, for example, in (i) the mechanism by which macrophages kill *Mtb* through both the innate and acquired immune induction of a vitamin D–dependent antimicrobial peptide–mediated pathway, (ii) the role of interleukin-32 (IL-32) in activating macrophages and generating cross-presentation of antigen to major histocompatibility complex (MHC) class I–restricted $CD8^+$ T cells, (iii) CD1 presentation of nonpeptide antigens to T cells, (iv) cytotoxic T cell release of the antimicrobial peptide granulysin, and (iv) the vitamin D dependence of autophagy of *Mtb*-containing vacuoles.

THE HUMAN IMMUNE RESPONSE TO TB

The innate immune system rapidly recognizes invading pathogens, mounting a direct antimicrobial response that is critical to TB control. The number of *Mtb* bacilli required to cause disease in humans is estimated to range from 1 to 400 bacilli (*43*), a number sufficiently small that, in many individuals, infection may be eliminated by innate responses without enough bacterial antigen load developing to stimulate T cell responses. Indeed, there is anecdotal evidence suggesting that some clinicians exposed for long times to active TB patients curiously remain tuberculin skin test (TST)–negative, which suggests that the innate immune response may be sufficient to control TB infection in some individuals. Yet, clearly, in others, adaptive immune responses are required for TB control. Understanding the interaction between innate and adaptive immune compartments in controlling TB in humans is thus critical for advancing new means to prevent and treat disease.

Pattern recognition receptors trigger distinct functional programs

Mtb lipoprotein interactions with Toll-like receptor 2 (TLR2) (*44*) have been found to trigger host responses, including the release of IL-12 (*45*), which is necessary for the generation of T helper 1 (T_H1) responses. Activation of TLR2 leads to the induction of IL-15 and components of the IL-15 receptor, triggering differentiation of monocytes into macrophages, with antimicrobial activity against mycobacteria (*46*, *47*). In addition, activation of TLRs on monocytes induces granulocyte-macrophage colony-stimulating factor (GM-CSF) and GM-CSF receptor, stimulating their differentiation into immature dendritic cells (DCs) with the capacity to release cytokines and efficiently present antigen through MHC class II to $CD4^+$ T cells (*46*).

In contrast to TLR2, which is expressed on the cell surface, nucleotide-binding oligomerization domain 2 (NOD2) is a cytoplasmic receptor belonging to the NOD-like receptor family. NOD2 mediates the response to peptidoglycan by sensing muramyl dipeptide (MDP) of *Mtb* (*48*, *49*), synergizing with TLR2 activation in triggering monocyte cytokine responses (*50*). Yet, the NOD2 and TLR2/1 pathways have different effects on subsequent immune responses. Activation of monocytes through NOD2 but not TLR2/1 induces IL-32 (*51*), a secreted protein without a known murine homolog. IL-32 triggers monocytes to release proinflammatory cytokines (*52*) and triggers an antimicrobial response to mycobacteria (*53*, *54*). Activation of monocytes through NOD2, by triggering IL-32 release, also results in the differentiation of monocytes into DCs, which efficiently present exogenous antigen through both MHC class II to $CD4^+$ T cells and MHC class I to $CD8^+$ T cells (*51*). The ability of macrophages to present exogenous antigen via MHC class I to $CD8^+$ T cells is known as cross-presentation and is an essential component of the initiation of $CD8^+$ cytotoxic T cell responses for host defense against infection and cancer (*55*). Therefore, *Mtb*-stimulated NOD2-induced IL-32 leaves DCs with the capacity to cross-present exogenous antigen via MHC I to $CD8^+$ T cells and better fight *Mtb* infection in humans (*51*).

Activation of macrophage microbicidal activity by innate immunity

Mtb has evolved the ability to prevent phagosome-lysosome fusion and elude one of the major microbicidal mechanisms mediated through reactive oxygen species. In mouse macrophages, antimicrobial activity has been shown to be largely nitric oxide–dependent (*56*, *57*). Although in human monocytes and monocyte-derived macrophages, nitric oxide synthase has been detected (*58*), antimicrobial activity appears to be nitric oxide–independent (*59*). Instead, human macrophage mycobactericidal activity has been found to be mediated by a TLR-induced vitamin D–dependent induction of antimicrobial peptides cathelicidin and DEFB4 (β-defensin 2). TLR activation leads to the induction of 25-hydroxyvitamin D3-1α-hydroxylase (CYP27b1), which converts 25-hydroxyvitamin D (25D) into the bioactive 1,25-dihydroxyvitamin D (1,25D), up-regulation and activation of the vitamin D receptor (VDR), and downstream induction of cathelicidin and DEFB4 (Fig. 1) (*60*–*64*). Cathelicidin, or LL37, is a 37–amino acid

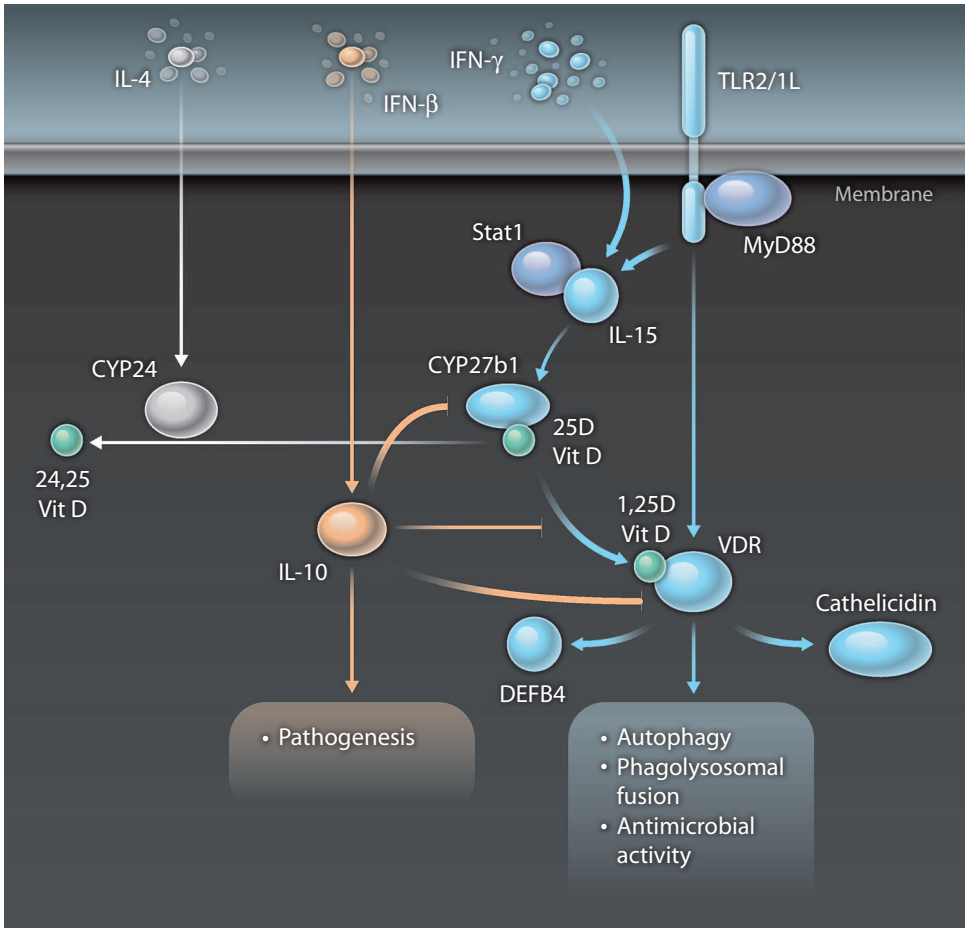

Fig. 1. Regulation of the vitamin D antimicrobial pathway by type I and II IFNs. A schematic diagram indicating that (i) TLR2/1 ligands and IFN-γ induce activate macrophages through distinct signaling pathways but converge on a common vitamin D–dependent antimicrobial pathway (blue); (ii) IFN-β, by induction of IL-10 (red), inhibits vitamin D metabolism and blocks the antimicrobial pathway; and (iii) IL-4 promotes vitamin D catabolism (gray), also blocking the vitamin D antimicrobial pathway.

amphipathic α-helical peptide, cleaved from a precursor protein, the cationic antimicrobial peptide (CAMP), that has the rare ability to co-localize with *Mtb* in the phagosome and can kill *Mtb* in vitro (*63*). Note that the CAMP homolog in mouse has no vitamin D response elements in contrast to three vitamin D–responsive elements in the human CAMP promoter region (*65*), consistent with the fact that mice are nocturnal animals and most vitamin D production derives from the ultraviolet B (UVB)–triggered photoconversion in the skin of 7-dehydrocholesterol to vitamin D.

IFN-γ induction of antimicrobial activity

Persons with compromised cell-mediated immunity (CMI) are at increased risk for TB (*66*). HIV-infected individuals with reduced $CD4^+$ T cells have increased susceptibility to TB, and those with the lowest $CD4^+$ T cell counts have the highest frequency of disseminated disease (*67*), clearly showing the importance of adaptive immune responses in protecting against the disease. A key function of $CD4^+$ T cells in this context is the production of IFN-γ: humans with Mendelian genetic disorders leading to the decreased production of, or response to IFN-γ, are highly susceptible to TB and other mycobacterial diseases (*22*–*24*, *68*). The ability of mycobacteria to induce secretion of ISG15 (IFN-stimulated gene 15), an IFN-inducible, ubiquitin-like modifier, appears critical for the production of IFN-γ. Individuals with a homozygous mutation in ISG15 display an impaired IFN-γ response and develop disseminated infection after BCG vaccination (*69*), although ISG15 may regulate other pathways.

The induction of IFN-γ is pivotal to an effective human immune response against *Mtb*, yet it has been puzzling that many studies of IFN-γ treatment of human macrophages in vitro have consistently failed to show killing of bacilli in *Mtb*-infected macrophages (*58*, *70*–*76*). It has even been concluded that "human macrophages infected with *Mtb* are desensitized to this cytokine" (*76*). However, experiments using physiologic concentrations of 25-vitamin D demonstrate that activation of monocytes/macrophages by either TLRs (*61*, *63*, *77*, *78*) or IFN-γ (*79*) can kill *Mtb* in vitro, and that both innate and acquired immunity converged on a common vitamin D–dependent antimicrobial pathway (Fig. 1). This antimicrobial response includes 25D-dependent induction of autophagy (*77*–*79*), phagolysosomal fusion, and the up-regulation of antimicrobial peptides (*63*, *78*, *79*). Together, the evidence suggests that acquired immunity against human mycobacterial disease may depend on IFN-γ production.

Contribution of vitamin D to host defense in TB

Because vitamin D production in humans is dependent on exposure to UV light, vitamin D deficiency is prevalent in dark-skinned populations, including individuals of African descent, who are known to have increased susceptibility to TB and other infectious diseases (*80*–*86*). There is evidence that African Americans are both more susceptible to TB infection (*84*) and contract more severe disease (*39*). Vitamin D–sufficient sera from white individuals can support TLR-induced cathelicidin mRNA (*63*) or IFN-γ–induced antimicrobial peptide expression, but serum with lower 25D levels from African Americans cannot (*79*). Supplementation with vitamin D in vitro restored their ability to do so. In addition, the IFN-γ–induced response, including antimicrobial activity, was dependent on serum 25D levels, with 25D-deficient serum able to support an antimicrobial response in vitro only after 25D was added (*79*).

A role for vitamin D in host defense in human TB is suggested by direct correlation of serum 25D levels with disease outcome. Meta-analysis of these studies suggests a 70% probability that a healthy individual would have higher 25D serum levels than an individual with untreated TB (*87*). Serum 25D levels were found to correlate with disease activity in Indonesian patients (*88*), Gujarati Asians in London

(*89*), and the co-susceptibility to TB/HIV infection (*90*). In addition, the seasonal variation in serum 25D levels was inversely correlated with the incidence of TB (*90*). As mentioned, VDR polymorphisms are known to predispose to TB, and the TaqI (*91*–*93*) and FokI polymorphisms (*93*, *94*) have been shown to exacerbate 25D deficiency (*89*).

There is a long history of using vitamin D to treat TB with "reported" success (*95*), although in fact, none of the studies can be considered definitive. A study in 1848 at the Hospital for Consumption and Diseases of the Chest, Brompton, UK, revealed that cod liver oil, rich in vitamin D, when provided as an adjuvant to then supportive therapy, improved the course of disease (*96*). In 1946, Dowling and Prosser Thomas reported the treatment of patients with cutaneous TB with vitamin D_2 (*97*). Of historical interest, Niels Ryberg Finsen received the 1903 Nobel Prize for Physiology or Medicine for curing cutaneous TB with UV light. Contemporary clinical studies have also indicated a potential benefit for vitamin D supplementation as an adjuvant to chemotherapy measuring various endpoints including clinical and radiological improvement (*98*–*100*), sputum conversion (*99*, *101*), and immune responses (*100*, *102*, *103*). However, these studies are generally believed to be inconclusive because of a number of study design flaws including inadequate vitamin D supplementation, insufficient power, and genetic variation within populations. More definitive studies are required to determine whether vitamin D supplementation is useful in the prevention of, or as an adjunct to chemotherapy in, TB.

$CD8^+$ T cell responses

Evidence from mouse studies suggests a role for $CD8^+$ T cells in protection against *Mtb*, but the effect is much less than that of $CD4^+$ T cells and they appear to act later in infection (*104*). Human $CD8^+$ T cells have also been shown to participate in host defense against *Mtb*, because $CD8^+$ T cell lines and clones that recognized *Mtb* antigens in vitro were found to lyse *Mtb*-infected macrophages in an antigen-specific manner and restrict the growth of *Mtb* in macrophages (*105*, *106*). $CD8^+$ cytotoxic T lymphocytes (CTLs) contain one or more microbicidal effector molecules in their cytotoxic granules, which are able directly to kill intracellular *Mtb*, including the human antimicrobial protein granulysin (*107*, *108*). Because $CD8^+$ effector memory T cells that produce granulysin also express surface TNF-α, they are depleted by anti–TNF-α immunotherapy for autoimmune diseases, which may contribute to reactivation of TB in these individuals (*109*). Granulysin has also been detected in $CD4^+$ T cells (*110*), and its expression in $CD4^+$ T cells has been suggested as a marker of TB in children (*111*). Adults with active TB have been shown to have significantly lower circulating granulysin in their plasma compared to healthy controls. After treatment, plasma granulysin levels in TB patients increased to levels comparable to healthy controls (*112*). Note that granulysin has no equivalent in the mouse, again suggesting that the study of humans may provide the greatest insights into mechanisms of host defense against *Mtb* in humans.

Kaufmann postulated that, in addition to their directly killing the bacteria, T cells are able to lyse highly infected macrophages and disperse the bacteria to other macrophages, which, if appropriately activated, would result in bacterial clearance (*113*). If not, mere killing of infected macrophages could lead to spread of infection in lesions. One such example may be CTLs that express FasL and induce apoptosis in infected macrophages without directly killing the intracellular bacteria (*107*). The induction of apoptosis in infected macrophages allows other macrophages to take up the apoptotic bodies containing bacilli in a process known as efferocytosis and deliver the bacilli to lysosomal compartments where they may be destroyed (*114*).

Innate lymphocytes

A number of T cell populations are thought to be part of the innate rather than the acquired immune response, in part based on the presence of conserved germline or canonical T cell receptors (TCRs) or TLRs. T cells bearing a γδ-TCR accumulate at the site of disease in mycobacterial infection (*115*), and the presence of mycobacteria-reactive T cells correlated with the clinical manifestations of TB, being greatest in individuals with strong CMI (*116*). These γδ T cells bear canonical TCRs, suggesting that they are part of the innate rather than acquired immune response, programmed to recognize nonpeptide antigens (*117*). The functional role of γδ T cells in mycobacterial infection has been expertly reviewed (*118*).

The human CD1 gene family consists of a small number of genes that are structurally related to the MHC class I genes (*119*); however, unlike MHC class I molecules, CD1 proteins are nonpolymorphic. The CD1a, CD1b, and CD1c molecules (known as group I CD1 molecules) are present in humans but not in mice (*119*, *120*). Different CD1 molecules present a universe of lipid-containing antigens to CD1-restricted T cells (*121*–*131*). A conserved TCR has been to shown to mediate CD1b presentation of mycolyl lipid antigen (*130*), suggesting that these T cells are part of the innate immune response. CD1-restricted T cells are thought to contribute to host defense against microbial pathogens (*107*–*109*, *123*) by secreting high levels of IFN-γ and low levels of IL-4 (*123*). In addition, mycobacteria-reactive CD1-restricted T cells typically show a high degree of cytolytic activity in vitro against antigen-pulsed $CD1^+$ DCs (*122*, *123*) and also recognize and lyse $CD1^+$ targets infected with live virulent *Mtb* bacilli (*107*). Additional alternative antigen processing pathways have been implicated in the immune response to *Mtb* including presentation of peptides by human leukocyte antigen–E (HLA-E) (*132*, *133*) and HLA-I (*134*).

A subset of $CD8^+$ T cells known as mucosa-associated invariant T cells (MAITs) recognize *Mtb*-infected cells, including DCs, macrophages, and epithelial cells (*135*). These *Mtb*-reactive $CD8^+$ cells express the Vα7.2 TCR, suggesting that they represent an innate T cell population. The *Mtb*-reactive MAITs are decreased in blood from patients with active TB and greatly enriched in human lung.

There has been a considerable advance in our understanding of how natural killer (NK) cells contribute to host defense against *Mtb* infection. The NK-activating receptor NKG2D has been shown to interact with ULPB1 on *Mtb*-infected monocytes and alveolar macrophages and contribute to lysis of these infected cells (*136*). IL-22 produced by human NK cells inhibits growth of *Mtb* by enhancing phagolysosomal fusion (*137*) and through the expression of calgranulin A (*138*). Parallel work in a mouse model indicates that $NK1.1^+$ cells and IL-22 regulate vaccine-induced protective immunity against *Mtb* (*139*). NK cells also regulate $CD8^+$ T cell effector function in TB, enhancing their capacity to lyse *Mtb*-infected monocytes (*140*). Although their role in TB is unclear, because NK cells are present in TB lesions, respond early in infection of *Mtb*, and are known to secrete IFN-γ, they may contribute to initiating innate and acquired responses.

The type I IFN pathway in human TB

Using gene expression arrays on peripheral blood of TB patients in Europe and in Africa, several laboratories have identified sets of genes that distinguish individuals with active TB disease from those with

latent infection (*141*, *142*). The most marked characteristic of the "signature" for active TB thus far is the increase in a set of genes regulated by IFNs, in particular the type I IFNs, IFN-β and IFN-α (*141*, *143*), although the IFNs themselves were not detected by the microarray. The induction of the type I IFN gene program was associated with the extent of disease (*141*) and resolved within 2 months of treatment (*144*). At the same time, studies have shown that *Mtb* induces type I IFN in macrophages, requiring the ESX-1 secretion pathway (*145*) for *Mtb* double-stranded DNA to gain access to the cytoplasm where it activates DNA sensors including STING (*146*). In a mouse model, the hypervirulent *Mtb* strain HN878 induced high levels of type I IFN expression and low levels of IL-12p40 (*147*, *148*) that augments T_H1 responses (*149*). One characteristic of the HN878 strain is a specific phenolic glycolipid (*150*, *151*); however, the ESX-1 secretion pathway of *Mtb* is involved in type I IFN induction (*145*) and inflammasome activation in macrophages (*152*).

Genetic studies indicating an essential role for innate immunity in host defense against mycobacterial infection

Studies of gene polymorphisms in humans with mycobacterial infection have provided definitive insights into the role of innate and acquired immunity in resistance versus susceptibility to disease, and provide independent confirmation of mechanisms found in in vitro studies. The most comprehensive study of TLR pathway gene polymorphisms in TB found single-nucleotide polymorphisms (SNPs) in TLR9 and TOLLIP to be associated with susceptibility and SNPs in TLR8 associated with resistance to disease (*153*). In addition, human NOD2 polymorphisms have been shown to be associated with susceptibility to TB (*154*). Similarly, in studies of rare Mendelian gene, deficiencies resulting in failure to produce or respond to IFN-γ led to very severe disease, establishing the role of IFN-γ as a necessary condition for protection (*22*–*24*).

VDR polymorphisms represent the strongest human common genetic variants known to predispose to TB, and the TaqI (*91*–*93*) and FokI polymorphisms (*93*, *94*) have been shown to exacerbate vitamin D deficiency (*89*). The vitamin D antimicrobial pathway clearly represents one antimicrobial mechanism against *Mtb* in humans (*155*), although there may be other mechanisms yet to be found in humans.

Learning from leprosy

There are inherent challenges in studying the dynamics of TB in lungs and other organs of humans. However, leprosy, caused by *Mycobacterium leprae*, offers an enticing model for investigating the regulation of immune responses to mycobacterial infection. Leprosy primarily affects the skin, facilitating the study of human immune responses at the easily accessible site of disease where the battle between the immune system and the pathogen is being fought. The disease affects half a million people, posing significant health, social stigma, and economic burdens on developing countries (*156*). Moreover, leprosy is a disease that presents as a clinical/immunologic spectrum (*157*), providing an opportunity to study the spectrum across resistance to susceptibility to disease. The fundamental insights into human immune responses gained from leprosy have provided useful paradigms for investigation of other cutaneous inflammatory diseases and have contributed to the development of immunomodulatory approaches for the treatment of skin disease.

At one end of the disease spectrum, patients with tuberculoid leprosy (T-lep) typify the resistant response that localizes the bacilli in organized granulomas and kills or restricts the growth of the pathogen. The number of lesions is few and that of bacilli is rare, although tissue and nerve damage are frequent. At the opposite end of this spectrum, patients with lepromatous leprosy (L-lep) represent susceptibility to disseminated infection. Skin lesions are numerous, and growth of the pathogen in macrophages is unabated. These clinical presentations correlate with the level of CMI against *M. leprae*, greater in T-lep versus L-lep patients. Conversely, anti–*M. leprae* antibodies are greater in L-lep than in T-lep patients. Most of the patients have a mixed form of the disease, borderline leprosy (BL), which has the potential to shift in a dynamic process toward the poles, called reversal reaction (RR), where patients, spontaneously or during chemotherapy, upgrade to the T-lep form, or erythema nodosum leprosum, which has immune complex pathology as they shift toward the L-lep pole.

The study of leprosy has provided a number of insights into the immunobiology of human innate and adaptive immunity. Early studies showed that the frequency of $CD4^+$ and $CD8^+$ T cell subsets at the site of disease correlates with the clinical form of the disease (*158*). Further studies identified human T_H1 and T_H2 cytokine patterns in lesions of a human disease for the first time, including the T_H1 cytokine IFN-γ in T-lep lesions and the T_H2 cytokine IL-4, as well as IL-10, in L-lep lesions, demonstrating that the pattern of expression correlates with clinical outcome (*159*–*161*). $CD8^+$ T cells in L-lep lesions were found to suppress T_H1 responses via secretion of IL-4 (*162*–*165*), thereby acting as regulatory T cells. What is more, the local cytokine pattern influences macrophage differentiation, leading to divergent macrophage functional programs, the IL-15–induced, vitamin D–dependent antimicrobial program in T-lep lesions, and the IL-10–influenced phagocytic program in L-lep lesions deficient in microbicidal activity (*47*).

Antagonistic roles of IFNs: Type I versus type II IFNs in leprosy

Considering the correlation of type I IFN expression with disease pathogenesis in TB, studying the spectrum of leprosy may help to elucidate the mechanisms by which type I IFNs may suppress IFN-γ–induced host defense responses. An integrative genomics approach revealed an inverse correlation between IFN-β and IFN-γ gene expression programs in lesions at the site of disease (*166*). The type II IFN, IFN-γ, and its downstream vitamin D–dependent antimicrobial genes were preferentially expressed in the lesions from patients with the self-healing tuberculoid (T-lep) form of the disease and mediated antimicrobial activity against the pathogen, *M. leprae*, in vitro. In contrast, the type I IFN, IFN-β, and its downstream genes, including IL-10, were induced in monocytes by *M. leprae* in vitro and were preferentially expressed in the lesions of disseminated and progressive lepromatous (L-lep) form. The IFN-γ–induced macrophage antimicrobial response was inhibited by IFN-β and IL-10 by a mechanism involving blocking the generation of bioactive 1,25D, as well as inhibiting induction of antimicrobial peptides cathelicidin and DEFB4 (Fig. 1). The ability of IFN-β to inhibit the IFN-γ–induced vitamin D pathway including antimicrobial activity was reversed by anti–IL-10 antibodies, suggesting a possible target for therapeutic intervention.

Integration of leprosy lesion gene expression profiles with blood profiles from TB patients

As mentioned above, a key study of gene expression profiles in peripheral blood from TB patients markedly found a differential expression of an IFN-inducible gene profile in patients with active versus latent disease (*141*). Integration of the training cohort from this study

with the peripheral blood mononuclear cell–defined IFN-specific genes revealed an IFN-β–induced gene program in active TB. Comparison of this cohort with a second independent active TB cohort (*142*) and the L-lep profile identified a common signature of 16 IFN-β–induced genes. Considering that IL-10 was identified in the IFN-β pathway in leprosy lesions, integrating the common IFN-β gene signature with the gene expression profile of IL-10–treated monocytes revealed that 9 of 16 IFN-β–induced genes in the leprosy and TB common signature are also downstream of IL-10. The common IFN-β/IL-10 signature was present even though (i) one study involved leprosy and two studies involved TB, (ii) one study involved skin lesions and two studies involved peripheral blood, (iii) the comparisons in each case were two different control groups, and (iv) all three studies used different microarray platforms. In addition, type I IFNs have been shown to contribute to disease susceptibility in TB by cross-regulating induction of IL-1 (*167*, *168*) and blocking IFN-γ–induced cytokine responses in monocytes (*169*). The data strongly indicate that a common IFN-β–inducible gene program correlates with extent of disease in both leprosy and TB, suggesting that IFN-β, through mediation by IL-10, is a common factor contributing to pathogenesis in the two distinct mycobacterial diseases. This signature currently represents the best correlate of progression from latent infection to active disease and as such is a marker of TB pathogenesis rather than protection (Fig. 2).

In the published work on peripheral blood gene expression profiles in TB, there was little evidence for increased expression of type I IFN–induced genes in peripheral blood from latently infected individuals compared with healthy controls, suggesting that there is likely to be a causal association between the IFN-β and IL-10 profiles and active disease and tissue damage. This raises the interesting possibility that in individuals who are able to maintain their *Mtb* in a latent or persistent state, a decrease in the type II IFN response or an increase in the type I response, perhaps induced by intercurrent viral infection, could shift the balance from latent to active disease (*170*).

THE DYNAMIC SPECTRUM OF HUMAN TB

Different forms of human TB have long been described (*39*, *171*). Although it has been clinically useful to dichotomize TB into active and latent infections, we suggest that TB, like leprosy, may be best understood as a dynamic spectrum of protective and pathogenic responses (*40*) that correlates with an immunological spectrum (Fig. 3). From an evolutionary standpoint, if the immune responses were fully effective in killing the pathogen, neither historically ancient disease would exist. When the T cell–mediated innate and acquired responses are compromised, as in HIV, TB often progresses more rapidly than in HIV-negative persons. XDR TB is more rapidly progressive than drug-susceptible TB in all patients, because treatment is usually inadequate. Because *Mtb* exists essentially only in human species, it has almost certainly evolved to persist in the human population, with only sufficient number of individuals developing major lung pathology to assure transmission and survival of the pathogen, the remainder being contained by immune responses that often allows the pathogen to persist but ensures the survival of the hosts past reproductive age.

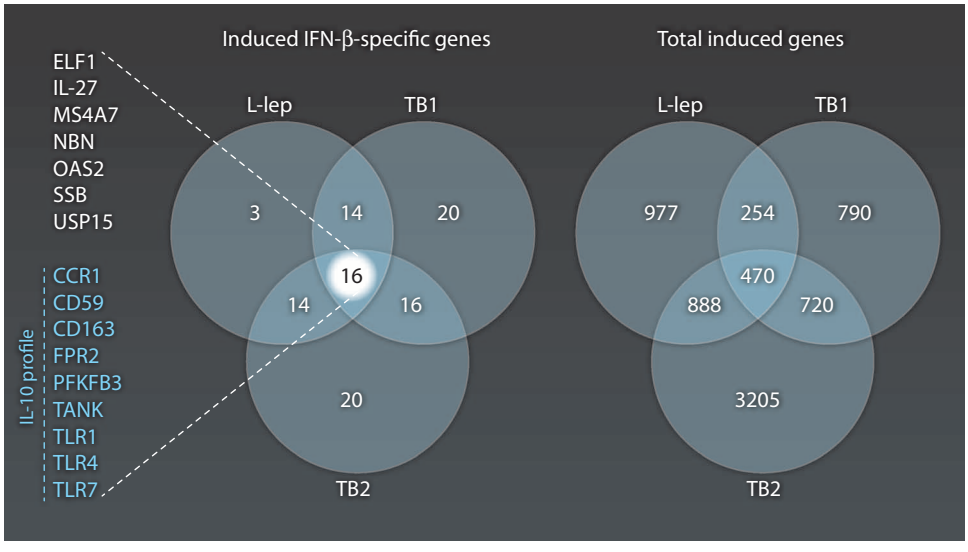

Fig. 2. Common type I IFN signature in the blood of active TB patients and L-lep lesions. (**A**) Dotted lines indicate either the expected fold enrichment of one (upper panel) or the hypergeometric enrichment *P* value of 0.05 (log *P* = 1.3, lower panel). Bonferroni multiple hypothesis test correction was applied for each group. (**B**) Venn diagrams show the total number of significantly ($P < 0.05$) induced genes and IFN-β–specific induced genes between L-lep, TB1 (UK cohort) (*141*), and TB2 (Germany cohort) (*142*) data sets. Enrichment analysis was performed to determine the overlap between IFN-specific genes and TB whole-blood transcripts associated with disease state [active (ACT), latent (LTN); fold change ≥ 1.5 and $P \leq 0.05$]. Hypergeometric distribution–based enrichment analysis was performed to determine significance of IFN-β genes induced in all three data sets ($P < 0.007$). Common genes between the three data sets were compared with genes induced by IL-10 in a human monocyte data set. Genes in red are also induced by IL-10, and this overlap with IL-10 is greater than expected (9 of 16 common IFN-β–specific induced genes, compared to 138 of 470 total common induced genes; hypergeometric $P = 0.02$) [reprinted with permission from Teles *et al.* (*166*)].

As illustrated in Fig. 3, one can relate the findings in the correlation between the pathological spectrum and the immunological spectrum in leprosy perhaps to explain the significant variation in the outcomes of infection with *Mtb*. At one end of the spectrum, there are individuals who have been repeatedly exposed to infection by TB patients, yet remain healthy and TST-negative. We would suggest that they may have sufficient innate immune responses to control the initial infection and prevent the input infection from growing to a sufficient number of organisms to generate an acquired T cell response, reflected in a positive TST. We believe that this will be an important group to study and could inform about the protective mechanisms of innate responses.

A small percentage of individuals infected with *Mtb* develop a rapidly progressive form of disease, which we suggest reflects an inability to develop appropriate innate or acquired protective responses. Most infected individuals, however, develop sufficient bacillary loads to generate a cell-mediated immune response, as determined by tuberculin skin testing or in vitro IFN-γ assays. This cell-mediated immune

response can control the infection in 90% of individuals, leading to a latent or persistent state in some unknown percentage. In a few individuals, chronic TB develops such that a single index case, with what is symptomatically no greater than a smokers cough, can infect several hundreds of individuals over time. In most of the individuals infected with *Mtb*, as in the case of BL, it is likely that there is a battle between protective, IFN-γ–mediated immune responses and an inflammatory response that contributes to tissue damage and pathogenesis. Rather than being a static disease, positron emission tomography scanning has shown dynamic changes in inflammatory reactivity of different lymph nodes in patients with latent or persistent infection over time (*40*). An estimated 10% of individuals who have been infected with *Mtb* and developed latency reactivate their lesions over their lifetime and develop clinical disease, which itself can present as a clinical spectrum. At one end, patients with tuberculous pleuritis are relatively resistant, able to mount a successful cell-mediated immune response, analogous to RRs in leprosy, where they develop an increased IFN-γ–protective response that limits the infection and restricts the bacillary load. Few or no bacilli are found in tuberculous pleuritis, with IFN-γ levels elevated in the pleural fluid relative to peripheral blood of patients (*172*). Vitamin D (bioactive 1,25D) levels are also increased in pleural fluid in tuberculous pleuritis patients, suggesting the vitamin D–dependent microbicidal peptide mechanism may be operative to limit the infection (*173*).

At the opposite end of the spectrum, disseminated or miliary TB is most similar to polar L-lep, often with large numbers of bacilli and foamy macrophages. Pulmonary and extrapulmonary diseases are manifestations of intermediate resistance. Extrapulmonary TB, unlike pulmonary TB, is not a major contributor to transmission. Although most pulmonary TB cases reflect a strong effort to contain the infection by granuloma formation, the bacilli can multiply and disseminate to the oxygen-rich apex of the lung, and the lesions develop as cavities with extracellular bacilli, which invade the bronchioles, thus enabling transmission of the infection to new hosts. It is unclear to what extent the tissue destruction in the lesions is due to the toxicity of the pathogen compared with that of the inflammatory immune response. Although little is known about the cytokine profile at the early lesions of pulmonary TB, the type I IFN profile was detected in the blood of patients with active pulmonary TB (*141*). In conclusion, these diverse manifestations of TB suggest that it may be useful to think of TB not as a single clinical disease but as a clinical spectrum very analogous to that of leprosy. We believe that the mechanisms described help to explain the evolutionary survival of both mycobacteria in the human host, their ability to generate protective cell-mediated responses in most individuals, and the necessity to create lung pathology to ensure its continued transmission.

A WORD ON VACCINES

BCG vaccine, discovered in 1908, remains the most widely used vaccine in the world, provided to about 104 million children annually. Yet, its protective efficacy against TB in adults has varied in clinical trials from 0 to about 80% in different populations (*174*). What BCG does appear to do effectively is to prevent miliary and disseminated disease in children. For many years, it was thought that the mechanism of protection was attributable to an acquired T cell response that blocked hematogenous spread of bacilli that initially seed the lower lung to the lung apices where cavities form. Thus, BCG was seen to prevent progression of infection to disease, not prevent infection.

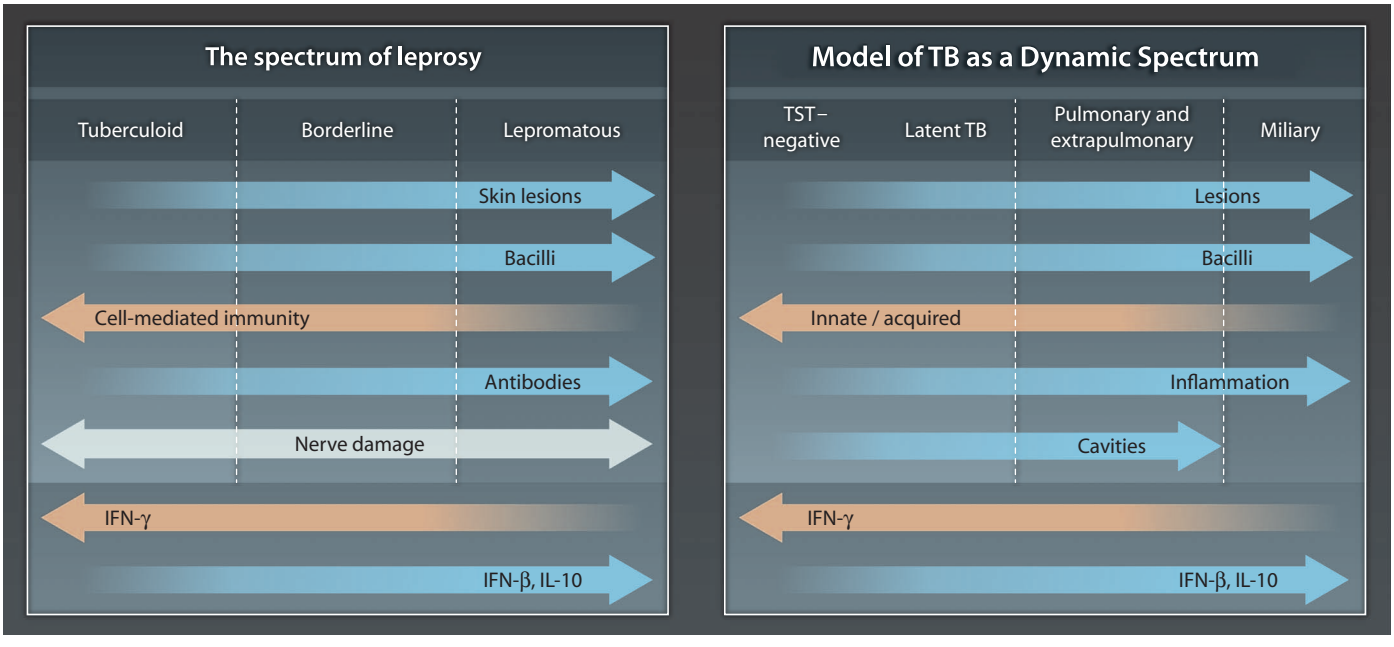

Fig. 3. The clinical and immunological spectra of leprosy and TB. (**Left**) Clinical and immunologic spectrum of leprosy, in which the clinical manifestations, including the ability to control the infection, correlate with the cytokine response in lesions. (**Right**) We suggest a parallel clinical and immunologic spectrum of TB. In between the dotted lines, in both the leprosy and TB spectra, we show what we infer to be the majority of patients, in which there are competing protective and pathogenic immune responses in disease lesions.

However, with recently developed IGRAs that can detect T cell responses to *Mtb* antigens not found in BCG, it is now possible to distinguish immunization with BCG from infection with *Mtb*. Unexpectedly, several studies have revealed a lower frequency of IGRA positivity in BCG-immunized individuals than in unimmunized controls, suggesting that BCG, and possibly newer vaccines, may, in fact, be capable of reducing risk of infection by *Mtb*, not just preventing the progression of infection to disease (*175*, *176*). BCG represents a standard treatment for superficial bladder cancer, and there is intriguing preliminary evidence that BCG immunization may have a significant therapeutic effect in multiple sclerosis (*177*) and in type 1 diabetes (*178*).

IMPLICATIONS

From the epidemiological perspective, the major objective in any infectious disease is to interrupt transmission. Traditionally, it was thought that the best way to accomplish that in TB was to provide appropriate treatment, which reduces transmission of drug-sensitive TB within weeks. With the emergence of MDR and XDR TB, and the current annual incidence decline of 2% even for susceptible TB, it will take many years to reduce the incidence by 50%, let alone reach the target of TB elimination—one case per million—urged by the World Health Organization (WHO) (*179*). A number of promising new drugs have been developed in the past decade; one of which was recently approved by the U.S. Food and Drug Administration (FDA) for use in MDR TB (bedaquiline), but effective combinations must be developed to prevent the emergence of resistance to the new drugs (*180*, *181*). However, even if there were effective new drugs that have targets in *Mtb* and could be used to treat both drug-susceptible and MDR and XDR TB, there remain enormous problems of cost, supply chain management to forecast the needs and deliver the drugs to the hospitals and clinics in high-burden developing countries, and potential adverse effects. For example, if the current costs for treatment of drug-susceptible TB with standard DOTS (directly observed treatment short-course) regimen vary between about $100 and $200 per patient (*112*), the cost of treatment of MDR patients ranges from $3500 to $7500 per patient, which in some countries would represent more than the entire annual health budget (*8*). In addition, a huge challenge with any long-term drug treatment—the 6 months required for drug-susceptible TB or 24 months for MDR TB—is patient compliance; after patients begin to improve clinically with treatment, they frequently discontinue treatment.

The BCG vaccine has been used for almost a century and has protected against disseminated TB and tuberculous meningitis in children, yet its protective efficacy in adults has varied between 0 and 80% in different trials (*179*). Clearly, a safe, effective, and affordable vaccine that could prevent either disease or infection would be of enormous benefit, especially in high-burden TB countries. We believe that understanding the dynamics of TB and leprosy and variation in individual responses to infection will require further elucidation of immunological mechanisms of protection and pathogenesis. For a pathogen such as *Mtb*, which lacks an animal reservoir and depends on creating lung pathology for transmission, from a public health perspective, the most important knowledge needed is how to interrupt the cycle of transmission. Although BCG vaccine has long been thought to prevent only disease after infection, because of the recent development of *Mtb*-specific IGRAs, there are suggestions that it may also reduce the frequency of infections (*175*). Intervention with either drugs or vaccines will require greater understanding of not only "correlates of pathogenesis" being elucidated by gene expression profiles of patients with active and latent infection but also "correlates of protection" currently lacking. To be able to delineate biomarkers of protection in TB will most likely require a vaccine that is at least 30 to 50% effective to identify those genes expressed in individuals who are protected from those who remain susceptible after vaccination to infection and disease. In both mycobacterial diseases in humans, leprosy and TB, we know some of the immune components that are *necessary* for protection, including innate immune responses triggered by *Mtb* ligands, which activate pattern recognition receptors, and acquired CD4 and CD8 T cells, IFN-γ, IL-12, TNF-α, and other cytokines. However, at present, we cannot define those mechanisms that are *sufficient* to assure protection.

From a variety of experiments, it is becoming increasingly clear that molecules able to stimulate autophagy and phagosome maturation may be important to create a hostile environment for intracellular mycobacteria that have mechanisms to block these host defense pathways (*76*, *182*–*184*). In this context, vitamin D seems to have a special capacity in humans to enable macrophage activation and production of microbicidal peptides and enhance autophagy (*77*–*79*). Vitamin D production is dependent on UV exposure, and melanin reduces the available UV activation of vitamin D production. In this context, many observations indicate that dark-skinned individuals have lower levels of vitamin D, suggesting that skin color may predispose individuals of African and Asian descent to greater susceptibility to infection and to more serious disease. It follows that vitamin D supplementation could enable such individuals to maintain adequate effector function of both innate and acquired immunity to eliminate the microbial invader, yet whether vitamin D supplementation in dark-skinned people can actually augment their resistance remains to be critically tested. This hypothesis is worthy of carefully designed randomized control clinical trials in vitamin D–deficient individuals and may be particularly relevant as well to populations in which new candidate vaccines are to be tested.

In leprosy, and we would suggest in TB, a delicate balance exists between protective and pathogenic responses. If initial aerosol infection doses are generally only a few bacilli, it is not unreasonable to believe that activation of macrophages through innate receptors may be sufficient to kill the input pathogens even before they are able to grow to a suitable antigenic load to engage the acquired immune response. Thus, the highest state of immunity may actually exist in exposed individuals who remain skin test–negative (*185*), and molecular studies of their innate responses could be very informative, yet we have almost no understanding of the early events of innate immunity in humans exposed to *Mtb*. Skin test conversion and in vitro production of IFN-γ may, in a sense, reflect a failure of the innate response to eliminate the invading pathogens, allowing them to multiply to an antigen level sufficient to expand T cells. In the lesions, a dynamic battle is ongoing between protective responses in macrophages induced by IFN-γ including up-regulation of antimicrobial peptides and other mediators compared with suppressive and proinflammatory responses mediated by IFN-β, IL-4, IL-10, and TGF-β. Interventions including drugs, adjuvants, or biologics could change the balance between protection and pathogenesis.

The urgent necessity raised by recent failure of the clinical trial of the first new vaccine in half a century against TB (*38*) to protect against infection or disease in infants, despite having provided some protection in mouse, guinea pigs, and nonhuman primate models of

TB (*34*–*37*), is to define protective immune responses in humans. The most informative approach to addressing the question of which immune responses are necessary and sufficient for protection compared with pathogenesis in human TB is probably a systems analysis of unstructured gene expression in the various phases and manifestations of the disease, best studied if there were a partially protective vaccine. However, in contrast to leprosy, studying early events in TB lesions is very difficult.

Although there is an increasing number of molecular signatures or biomarkers for progression of latent TB to active disease (*141*, *143*), which are really correlates of pathogenesis, there is at present no established molecular signature for protection against infection or disease. We believe that in the absence of molecular signatures for the dynamic states of the infection that enable prediction of the course of disease and effects of interventions on correlates of protection, it may be necessary to think about developing a safe live infection challenge model that could be used in small-scale studies to measure ability to kill the pathogen (*186*). This has been a very important tool in studying protection in other infectious diseases, such as malaria and cholera. There have been preliminary studies using intradermal BCG as a live challenge and measuring bacterial survival after excision (*187*). With a genetically attenuated *Mtb* strain, such as an auxotroph unable to grow without essential nutrients, and with a conditional promoter, such as *tet-on*, allowing growth only in the presence of doxycycline, assuring that the challenge would die when the drug is removed, one could potentially create a very safe challenge strain. For a skin challenge, ideally, it should also express fluorescent or bioluminescent markers enabling simultaneous quantification of cell number and viability, which would enable, in a noninvasive local challenge model, measuring the range of individual differences both in innate immunity and in responses to adjuvants or vaccine candidates that lead to killing of the challenge strain. Or, if such an attenuated strain could be engineered to produce a product that could be measured systemically with high sensitivity and safety, such as a secreted product, it might be possible to have direct assessment of the ability to kill the challenge after aerosol delivery. If a preventive vaccine were able to increase killing of the challenge in small numbers of individuals, this would provide an important opportunity to characterize changes in gene expression in detail in those protected and those not and enable a decision on whether to expand to larger clinical trials. Conversely, without a preventive intervention that succeeds in increasing microbicidal activity, it will be very difficult to define precisely the most import knowledge gap in the immunology of these diseases—meaningful biomarkers or correlates of protection.

SOME BARRIERS AND CHALLENGES

TB is a complex and dynamic disease. There are numerous barriers to achieving adequate control of drug-sensitive TB and even greater complexities and costs to controlling MDR and XDR TB. Some of the present

Table 1. Some barriers to prevention and treatment of TB in developing countries.

Category	Barrier	Approach to overcoming barrier
Tools	Lack of an effective preventive or therapeutic vaccine	Research and testing of new candidates
	Lack of infection control and ventilation	Investments in hospital infection control
	Lack of rapid, inexpensive, point-of-care diagnostic	Research in new technologies
	Lack of access to drug susceptibility testing	Wider use of GeneXpert and new technologies
		Strengthening laboratory capacity
	Need for shorter regimens	New drugs, regimens, and clinical trials
Development of new drugs	Limited number of new drug targets and new drugs	Basic research on defining new drug targets in *Mtb*
	Need to test new drugs in combinations with existing regimens	TB Alliance and FDA agreement to test new drugs in combination regimens, not individually
	Dependence on surrogate endpoints, such as time to sputum clearance, rather than disease endpoints	Research on better correlates of infection, disease, and pathogenesis
		Research on live challenges
Health systems problems	At a country and global level, lack of IT, drug forecasting, central purchasing for many drugs, and supply chain management	Greater investments in health information systems in countries, training in modeling, forecasting, and supply chain management
	Limited number of producers and competition in second-line drugs	Linking TB drug purchase and supply to HIV and other drugs, subsidize second-line drugs
	Complexity of treatment of TB and HIV in co-infected individuals	Linking TB and HIV services in countries
	Organization of community-based care after hospitalization	Will require rethinking the role of hospital care and changes in health systems to improve outpatient treatment and follow-up
Funding	Limited availability of funds for basic and translational research and health systems	Greater contributions from countries, bilateral and multilateral donors, and international agencies, such as WHO and Global Fund to Fight AIDS, Tuberculosis and Malaria

limitations, as outlined in Table 1, are scientific, namely, the need for new and better tools such as vaccines and diagnostics. The Xpert MTB/Rif is a major diagnostic advance that can determine whether, in a sputum sample, there is drug-susceptible or MDR *Mtb* within 2 hours. However, its use is currently limited to major clinical centers, and there remains a need for a point-of-care diagnostic and drug susceptibility test. There is the urgent need for new drugs and regimens that can treat classical susceptible and drug-resistant TB. The challenge here is discovering drugs that not only affect a specific target but also can enter into the macrophage and its phagocytic vacuole and can kill latent TB organisms that may not be dividing and extracellular bacilli in necrotic lesions. Because of the small market primarily in poor countries, regrettably, many major pharmaceutical companies are dropping out of screening for new antibiotics for TB. Nevertheless, there have emerged a small number of promising new drug candidates and regimens that are in clinical trials (*181*).

Even if we had new and effective drug regimens, there are skeptics who would argue that we still could not get them to the patients in developing countries who need them. There are difficult barriers to providing access to treatment, outpatient care, and compliance with treatment issues in high-burden countries that can only be addressed at the level of changes in the health systems for TB themselves, including better health information technology (IT), forecasting drug needs to prevent stockouts, and provision of outpatient care and better supervised treatment.

There is another set of barriers that limits our ability to develop effective vaccines, some of which are summarized in Table 2. The most challenging relate to our lack of basic understanding of the nature of protective immune responses and, conversely, the role of the immune system in causing pathologic inflammation and tissue damage. Clearly, there is the need for testing candidates in the existing animal models, despite their known limitations as perfect models for human TB. However, testing vaccines for efficacy against TB in humans can only practically be done if we have appropriate correlates of protection that can be measured rapidly, rather than in perhaps decades, to see the effects on TB incidence in populations. In addition, any vaccine testing beyond phase 1 for safety will essentially have to be done in disease-endemic countries. That will require investments in creating multiple epidemiological trial sites, sustaining that infrastructure even when there is no vaccine to be tested, and developing clinical immunology laboratory capacity to learn from those trials.

Overcoming all those barriers will require more funding than is presently available or envisioned in the near future. At the present time, funding for TB research, particularly translational research on TB, is severely limited. The best estimate is that $4.6 billion is needed to mount a serious attack on the global TB problem, but that the current resources reveal a $1.6 billion gap between what is needed and what is available globally (*188*). The Global Fund to Fight AIDS, Tuberculosis and Malaria spends over 3.5 times more on HIV/AIDS and twice as much on malaria than it does on TB (*189*). The National Institutes of Health spent a tenth of what it funds for HIV/AIDS research on TB (*190*).

We believe that the simplistic view that there is "TB or not TB" is no longer the question. The scientific community urgently needs to

Table 2. Some barriers to development of effective vaccines against TB.

Category	Barrier	Approach to overcoming barrier
Basic immunology	Understanding necessary and sufficient mechanisms of protection, for example, roles of innate immunity, acquired immunity including $CD4^+$ and $CD8^+$ T cells, T_H17 cells, NK cells, B cells, and neutrophils, and cytokines in protection and pathogenesis	More basic research, particularly on human immune responses
	Biomarkers or correlates of pathogenesis	Comparison of active, latent, and uninfected gene expression profiles
	Correlates of protection	Comparison of gene expression profiles in protected and susceptible individuals in trials of partially effective new vaccines
	Limitations of animal models in predicting human responses	Develop better animal models, such as humanized mice, with more human macrophages, genetically modified mice, and more detailed characterization of immune responses of nonhuman primates in TB
	Develop a safe human live *Mtb* challenge system	Create bioluminescent viability marker strains or secreted product markers in BCG or genetically attenuated *Mtb* strains that can be noninvasively monitored in humans
	Defining protective antigens	Basic research in animal models and human in vitro studies
	Distinguishing infection by *Mtb* in vaccinated individuals	If not essential for protection, exclude ESAT-6 and CFP10 antigens from vaccine so that IGRA assay can be used to ascertain TB infection
	Development of appropriate delivery systems that stimulate protective immunity and decrease priming for inflammatory responses	Basic research in animal models and clinical studies in humans
Clinical research	Developing clinical trial sites in high endemic areas where new vaccines can be tested	Training, funding, and linkages with TB laboratories
	Developing clinical research capacity in those sites	Training, funding, and linking with HIV and TB laboratories
Funding	Limited funding for basic research and especially human immunology of TB	Greater funding for both basic and clinical research in TB

develop new tools to contain the problem of multidrug resistance, reduce the incidence of classical TB, and develop better diagnostics and more effective vaccines. It has to partner with colleagues and institutions in the high-burden TB countries to strengthen the capacity to learn what is required to protect against this disease. Also, it critically needs funding for research, training, infrastructure development, and health systems strengthening if we are going to achieve the goal of elimination of TB, historically the largest infectious cause of death in the world, as a major public health problem.

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Editor's Summary

Tuberculosis's Next Top Model

In this state of the art review, Modlin and Bloom describe how the study of tuberculosis in humans is essential for understanding the complex dynamics of disease, as well as for developing better treatment and prevention strategies.

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