The currently available vaccine for tuberculosis (TB) is ineffective in developing countries. We need to understand the pathogenesis of TB in those countries and how it differs from the pathogenesis of TB in wealthy countries, to facilitate the design and interpretation of clinical trials of new vaccine candidates that are now available. We show here that these geographical differences parallel the strikingly different immunology and bacterial growth curves seen in animal models after high-dose and low-dose challenge with *M. tuberculosis* (Mt). We consider this point in the light of recent insights into the multiple pathways used by the immune response to control *M. tuberculosis* and the susceptibilities of these pathways to regulation and suppression. There are important implications for the screening, testing, and likely success of vaccine candidates.

The only currently licensed vaccine against tuberculosis (TB), bacille Calmette-Guérin (BCG), has been shown to protect against all forms of TB in clinical trials performed in northern developed countries [1]. In contrast, BCG has failed to protect against adult forms of TB in developing countries, especially in low-lying areas within 30° latitude of the equator [1]. Unfortunately, these are the countries in which most of the TB burden is found and where the need for a vaccine is greatest, so there is an urgent need to understand the geographical differences in the pathogenesis of TB. An analysis of the immunology and bacterial growth curves seen in animal models after high-dose and low-dose challenge with *Mycobacterium tuberculosis* suggests an illuminating parallel with the human data.

**ANIMAL MODELS**

First, we point out that there are 2 entirely different types of animal models, which are accompanied by different immunology and different bacterial growth curves. When specific pathogen–free (SPF) mice (BALB/c or C57Bl) are infected by aerosol with 100–200 living *M. tuberculosis*, the organisms proliferate for ~3 weeks until a Th1 response develops (interferon [IFN]–γ and tumor necrosis factor [TNF]) [2]. From then on, the number of viable bacilli in the lungs reaches a plateau. The animals eventually die, but this is due to expanding granulomas and cellular infiltration, and in effect the animals drown (shown diagrammatically in figure 1A).

This differs from what happens when mice are infected with a high challenge dose of *M. tuberculosis* (figure 1B). When >1 × 10^5 *M. tuberculosis* are administered, either directly into the airways (intratracheal injection) [3, 4] or by intravenous injection [5, 6], a similar plateau is reached at 3 weeks; however, after a further 10–14 days, bacterial proliferation starts again, and, in addition to granulomas, there is widespread pneumonia containing *M. tuberculosis*. It is the extent of this pneumonia, rather than of granulomas, that correlates with death [3].

Importantly, this secondary growth of bacilli in the high-dose challenge models is preceded by the appearance of lymphocytes expressing interleukin (IL)–4 [3–5]. However, the role played by IL–4 is controversial, for 2 reasons. First, high-dose challenge with mycobacteria is known to push the response toward Th2 [7], but this does not prove that the IL–4 is involved in the pathogenesis of TB. Second, IL–4 is not involved in the pathogenesis observed in the low-dose challenge model [2]. C57Bl mice lacking functional IL–4 and IL–13 genes [2] and BALB/c mice lacking signal transducer and activator of transcription (STAT)–6 are no less susceptible to low-dose aerosol challenge, and the bacterial growth still reaches a plateau. Nevertheless, it has now been
proven that IL-4 is directly involved in the progressive phase of late bacterial proliferation after high-dose challenge in BALB/c mice. In high-dose models, infection is attenuated in mice with nonfunctional IL-4 genes (IL-4/−/−) [3], and it can be partially treated by administering neutralizing antibody to IL-4 [6]. Interestingly, in wild-type mice the high-dose infection is accompanied by a marked increase in levels of transforming growth factor (TGF)−[3]. However, in IL-4/−/− mice there is a brief early peak in TGF−, which then falls to very low levels [3]. This raised the possibility that neutralizing the TGF− would also treat the disease after high-dose challenge [8]. Administering recombinant soluble type 3 TGF− receptors attenuated the disease and reduced the expression of IL-4 [8]. Thus, these 2 cytokines are interdependent in this model, and neutralizing either TGF− or IL-4 down-regulates the other. Interestingly, neutralizing IL-4 or TGF− does not cure TB in the mice, but it does reduce the bacillary load to a plateau resembling that seen after low-dose challenge (figure 1B).

**DAMAGING ROLE PLAYED BY Th2 CYTOKINES AND TGF−**

There are good reasons why this combination of high levels of Th2 cytokines and TGF− would be expected to facilitate a secondary phase of bacterial proliferation, even in the presence of an established Th1 response. *M. tuberculosis* inhibits classical Th1-mediated macrophage activation, because it blocks phagosome maturation [9], lysosome fusion [10], presentation via major histocompatibility complex class I [11], and triggering via the IFN− receptor [12]. Therefore, in the progressive phase of the disease, the immune system depends on several mechanisms that circumvent the failure of the infected phagosome—generating new phagosomes, promoting its uptake by a fresh macrophage, or directly exposing the bacteria to bactericidal peptides. These mechanisms are illustrated in figure 2 and include cytotoxic T lymphocytes [13], apoptosis, and autophagy [14]. Figure 3 illustrates how all of these mechanisms are susceptible to down-regulation by a combination of Th2 cytokines and TGF− [14–16]. IL-4 and IL-13 signal through the receptor IL-4Ra, which forms a heterodimer either with the γ common chain (for IL-4) or the receptor IL-13Ra1 (for IL-13) [17]. Ligation of these receptor complexes results in signaling via 2 different pathways. First, signaling via insulin receptor substrate–1 and Akt activates the serine/threonine kinase mTOR (mammalian target of rapamycin), which inhibits autophagy [14]. This is likely the reason why blocking Akt inhibits the proliferation of *M. tuberculosis* inside macrophages [18]. Second, IL-4, acting via STAT-6, up-regulates Bcl-2, which in addition to inhibiting apoptosis sequesters beclin-1, without which autophagy cannot be initiated (reviewed and discussed in 14). Other relevant pathways include increased expression of transferrin receptors and DC-SIGN, decreased expression of TNF, and increased expression of soluble TNF recep-
Macrophage activation by Th1 cells

Phagosome fails to mature
Lysosomes fail to fuse
→ Macrophage unable to kill M. tuberculosis

Additional mechanisms that circumvent the compromised phagosome

Apoptosis
Cytotoxic lymphocytes
Autophagy

Take up by new macrophage
Bactericidal peptides (e.g., granulysin)
New phagosome in same cell

All inhibited by IL-4, IL-13 and secondarily induced TGF-β

**Figure 2.** Effector mechanisms that kill *Mycobacterium tuberculosis*. The bacilli are taken up by macrophages that Th1 lymphocytes attempt to activate. However, the organisms inhibit phagosome maturation, lysosome fusion, and interferon (IFN)-γ–mediated activation and so block the classical killing mechanisms of activated macrophages. The immune system circumvents this block by means of 3 processes that can cause the ingested organisms to be taken up into new phagosomes or directly killed by microbicidal peptides from cytotoxic T lymphocytes. However, these 3 escape mechanisms—apoptosis, cytotoxic T lymphocytes, and autophagy—are all inhibited by Th2 cytokines and transforming growth factor (TGF-β).

tor, IL-10, and TGF-β. These are well-documented consequences of alternative activation of macrophages [19, 20]. Similarly, IL-4, IL-13, and TGF-β will tend to deviate the recruitment of T cells away from Th1 and cytotoxic T cells and toward Th2 and regulatory T cells [15, 16, 21]. Although TGF-β is also involved in driving Th17 cells [22], which might play some protective role in TB [23], this effect of TGF-β is strictly dependent on the other cytokines present [22], and high levels of TGF-β in the cyto-

**Figure 3.** Pathways that enable interleukin (IL–4) (and IL-13) together with secondarily induced transforming growth factor (TGF–β) to inhibit apoptosis, cytotoxic T lymphocytes (CTLs), and autophagy—the “escape mechanisms” described in figure 2. The IL-4 receptor signals via both signal transducer and activator of transcription (STAT)–6 and insulin receptor substrate (IRS)–1. Relevant mediators whose levels are increased are marked with “(+),” whereas those whose levels are decreased are indicated by “(−).” In addition to direct effects on the infected macrophage, IL-4 (together with TGF-β) also acts on T cells to bias differentiation away from Th1 and CTLs and toward Th2 and regulatory T (Treg) cells. These pathways are referenced in the main text. iNOS, inducible NO synthase; mTOR, mammalian target of rapamycin; sTNFR, soluble TNF receptor; TLR, Toll-like receptor; TNF, tumor necrosis factor.
The environment seen in TB are associated with progression [24].

**HIGH-DOSE CHALLENGE DUE TO OVERCROWDING AND DELAYED DIAGNOSIS**

These issues are equally relevant to humans, particularly in developing countries. We have pointed out previously that, although expression of IL-4 is increased in some patients with TB even in northern wealthy countries [25, 26], TB accompanied by very high levels of IL-4 is characteristic of the areas within 30° latitude of the equator, where BCG fails [27]. Sometimes TB in developing countries is accompanied by such high levels of IL-4 that it can be measured free in the serum by ELISA [27, 28]. Similarly, the expression of TGF-β and of its receptors in the blood or lungs of persons with TB is well established [24], and peripheral blood cells from patients with the most advanced TB show the highest release of both TGF-β and IL-4 [28]. It is therefore logical to suggest that much of the TB occurring in these areas is due to high-dose challenge, leading to high IL-4 and TGF-β levels, exactly as found in mice. This is indeed probable, because overcrowding and delayed treatment of open TB cases in developing countries will inevitably lead to prolonged exposure of family members sharing the same hut or room. Unfortunately, little is known about the actual numbers of bacteria that are released by coughing. One study counted infectious particles coughed directly into a sampling apparatus [29]. However, the bacterial counts in these particles were not assessed [29], and the method was unable to detect organisms in room air, although earlier experiments channeling air from a TB ward into guinea pig cages proved their presence [30]. Thus, we know neither the numbers of organisms released nor the number required to cause progressive disease in a partially immune human. Interestingly, it has emerged that many of the organisms in sputum are in a dormant state, leading to the possibility that they might accumulate in that state in a close contact before an unknown event causes their reactivation [31].

**PARTIAL IMMUNITY INDUCED BY ENVIRONMENTAL MYCOBACTERIA**

There are other reasons why TB occurring in developing countries is likely to be due to high-dose challenge. Saprophytic mycobacteria are ubiquitous, but the density of environmental contamination with mycobacteria and the lifestyle of the individual influence the degree of sensitization to them. For example, using identical techniques in Malawi and the United Kingdom, it was shown that immunization by a range of environmental mycobacteria was much greater in young Malawians [32]. Even in the United States, sensitization detected by skin testing is highest in agricultural workers in the humid southern areas and is lowest in US-born middle-class white persons in northern areas [33]. In humid developing countries, >90% of the population has a positive skin-test response to environ-
mental mycobacteria by the age of 15–20 years [1]. There is good evidence that this sensitization provides some protective effect against M. tuberculosis [34]. Therefore, higher challenge doses of M. tuberculosis are likely to be necessary to cause progressive disease [35] than would be the case for mycobacteria-naive individuals living in northern inner cities in the United States [36]. This requirement for a higher challenge dose to overcome partial immunity is routinely observed for all infectious diseases and was documented experimentally in guinea pigs with M. tuberculosis [35]. It is also likely to explain the requirement for high-dose challenge in certain mouse models of TB in developing countries, where preexposure to environmental mycobacteria has been demonstrated [37, 38]. (These models badly need to be properly defined in terms of doses and species of mycobacteria that are encountered in the breeding rooms, and such work is in progress.)

However, in developing countries there is parallel priming of a Th2 (IL-4) response to mycobacteria [32, 39, 40], possibly because of the simultaneous presence of helminths [41]. As pointed out above, it is known that high-dose challenge with mycobacteria drives an IL-4 response [7], but clearly this tendency will be exaggerated in individuals exposed to high challenge doses of M. tuberculosis if a Th2 response has been primed by the environment. Moreover, malnutrition [42], smoking [43], and psychological stress [44], all of which are prevalent in developing countries, contribute to the bias toward a Th2 response [42–44]. The malnutrition will often include vitamin D deficiency, which could further increase susceptibility [45]. These factors, which are summarized in figure 4, can all act independently of HIV infection, although infection with HIV will aggravate the situation.

CONCLUSIONS, LIMITATIONS, AND IMPLICATIONS

In conclusion, it seems probable that most individuals in developing countries, where BCG fails, are partially immune to TB as a result of BCG vaccination and sensitization by environmental mycobacteria [34]. Such sensitization might be able to protect them from low-dose challenge, and we suggest that this accounts for much of the efficacy of BCG in northern wealthy countries. However, in developing countries many people are malnourished, smoke, are stressed, and have a background Th2 response to mycobacteria already primed by the environment. In such people, prolonged high-dose challenge from untreated individuals sharing the same confined space might be able to drive enough IL-4, IL-13, and TGF-β production to overcome the protective response, resulting in the characteristic “high–IL-4” TB seen in many developing countries [27, 28]. Clearly, both types of TB will occur in all areas, but the bias toward high–IL-4 TB will be greater in developing countries.

A limitation of this analysis is the fact that a valid comparison of the effects of high-dose and low-dose challenge in mice is possible only with experiments involving M. tuberculosis H37Rv, which has been used repeatedly in both types of models with appropriate manipulations of IL-4 [2, 3, 6]. Clearly, it is possible that other strains of M. tuberculosis cause disease by different mechanisms. Nevertheless, the relevance of this simple concept derived from the H37Rv models to TB in developing countries should be investigated urgently, because it has major implications. First, models used to select vaccine candidates might need to include high-dose challenges of partially immune animals, to mimic the situation in the countries in which a new vaccine is most needed. (Standardization of such models is urgently required.) Second, it might be useful to document the immune response patterns (i.e., low–IL-4 TB versus high–IL-4 TB) in clinical trial areas—and, in particular, in trial participants who develop the disease. This will enable testing of the prediction that vaccinated individuals who develop TB tend to have the high–IL-4 pattern of response and will provide indirect support for the hypothesis that it might be very difficult to vaccinate against TB resulting from high-dose challenge in partially immune individuals.

References