

The immune responses against MTB in different geographic regions differ; thus, the protective vaccines for one region may not be useful in another



Patched-up – Anne Rook
Inkjet print of digital image
(29 x 32 cm)

'I am those that are no more. For no good reason
I am in the evening sun, those vanished person'
All Our Yesterdays (Poem) – Jorge Luis Borges

Anne Rook
Paris, France

Anne Rook has exhibited extensively in Britain, France, and Japan. She has also produced several artists' books, a number of which are in the Victoria and Albert Museum, London. Among her publications are: *Prints Now*, Gill Saunders and Rosie Miles, V & A Publications, 2006; and 'Sunek/Thrust', 26th Biennial of Graphic Arts, Ljubljana, 2005.

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Her main group exhibitions were 'Forest', Artislong Gallery, Kyoto (2006); Itami Museum of Arts & Crafts, Itami, Japan (2006); International Biennale of Graphic Arts, Ljubljana (2005); 'Rien à déclarer' Bordeaux, curated by Steve Williams (2004); 'Garden of Earthly Delights', curated by Indra Khanna, Brockwell Park, London (2004); and 'Riverside' East International, Norwich (2000).

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CHAPTER 28

Tuberculosis in Developing Countries due to High-Dose Challenge in Partially Immune Individuals

Graham AW Rook

Introduction

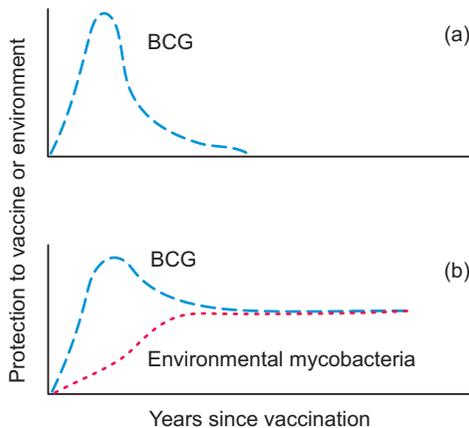
The only currently licenced vaccine against TB—the BCG—was shown to protect against all forms of TB in clinical trials performed in developed countries in the northern hemisphere. By contrast, BCG appeared not to protect against adult forms of TB in developing countries, many of which are situated within 30° latitude of the equator. Unfortunately, these are the countries where most of the TB burden is found, and where the need for a vaccine is greatest. We need to understand the pathogenesis of TB in developing countries, and the ways in which it differs from TB in rich countries, in order to facilitate the design of clinical trials with the new vaccine candidates that are now available, and the interpretation of their results. Indeed we might need to consider different types of vaccine for different populations and environments. We present here an account of the ways in which immune systems in rich and developed countries differ, and discuss how this might affect the prospects for vaccines that work in the poor and developing countries where they are most needed.

Effects of the Environment on Immune Systems in Developing Countries

Th1 Responses Induced by Environmental Mycobacteria

Saprophytic mycobacteria are ubiquitous, but the degree of sensitization to them that occurs is influenced by the density of environmental contamination with mycobacteria, and the lifestyle of the individual. For example, using identical techniques in Malawi and the UK, it was shown that immunization by a range of environmental mycobacteria was much greater in young Malawians (1). Even within the USA, sensitization detected by skin-testing is highest in agricultural workers in humid Southern areas, and lowest in middle class USA-born Caucasians residing in the North (2). In humid developing countries, more than 90 per cent of the population has a positive skin-test response to environmental mycobacteria by the age of 15–20 years (3). There is good evidence that this sensitization has some protective effect against MTB (4). Thus, part of the apparent failure of BCG in developing countries is in fact a statistical artefact due to the increasing protection derived from contact with environmental mycobacteria that narrows the gap between the incidences of TB in the vaccinated and non-vaccinated populations (Figure 28.1).

Figure 28.1 Two Interpretations of the Failure of BCG Vaccine to Protect against Adult TB in Developing Countries



Note: Figure 28.1a assumes that there is a peak of protection that wanes with time. Figure 28.1b suggests that although the protective effect of vaccination might wane slightly, it is probably maintained by boosting from environmental mycobacteria (dashed line). Moreover, the same environmental mycobacteria induce some protective response in non-vaccinated individuals (dotted line) so that eventually there is no difference between the vaccinated group and the controls. Unfortunately, this protection, whether involving BCG or not, is inadequate protection under developing country conditions, which are outlined in later sections.

Priming of Th2 Responses to Mycobacteria in Developing Countries

However, in developing countries there is parallel priming of a Th2 (IL)-4 response to mycobacteria (1, 5, 6), possibly because of the simultaneous presence of helminths. For instance, when PBMC from children in Cameroon's Sanaga valley were cultured for two days with PPD, IL-4 output increased linearly with increasing age up to 16 when it reached 50–60 pg/ml (5). We will discuss the geographical distribution of TB accompanied by high IL-4 levels, and the implications for pathogenesis, in a later section of this chapter.

The Role of Helminth Infections

It is reasonable to implicate some helminths in the induction of Th2 and of transforming growth factor (TGF)- β responses to mycobacterial antigens, and in consequent increased susceptibility to the disease. A marked correlation was found between total serum IgE levels and the incidence of TB in the various subdistricts in Cape Town (7). Second, total IgE and *Ascaris*-specific IgE levels were high in TB patients, and total IgE declined following successful treatment of TB (8, 9).

In Brazil, a higher prevalence of intestinal nematodes was seen in patients with TB compared to a matched control group: 57.8 per cent versus 20.9 per cent (10). In a study in Ethiopia, the most prevalent helminth infection was *Ascaris lumbricoides*. This was present in 53.5 per cent of TB patients, but only in 19.8 per cent of controls selected from the same households; so poverty and overcrowding should not be confounders (11).

In a cohort of HIV-infected adults in Uganda, eosinophilia, which is associated with a type 2 cytokine profile, correlated with subsequent progression to active TB (12). This progression was also associated with IL-5 and IL-10 responses to mycobacterial antigen (13). The authors then sought to explain the Th2 bias in terms of helminth infection, and found evidence that *S. mansoni* infection facilitates progression to active TB among HIV-1-infected Ugandans (14). Interestingly, gastrointestinal nematodes (frequently *Strongyloides stercoralis*, a nematode that lives in the mucosa of the small intestine) were equally common but not associated with TB (14).

It seems possible that the critical point is whether the parasites involved pass through the lungs, or have a life cycle that results in deposition of worm-derived adjuvant material in the lungs and in the lymph nodes that drain the respiratory system. Thus *Ascaris* and *Schistosoma* seem most frequently associated with susceptibility to TB, whereas often intestinal parasites are not.

Regulatory T Cell Function in Developing Countries

The helminths and other environmental organisms to which the populations of developing countries are exposed are also relevant for another reason:

they induce immunoregulatory circuits. People in developed countries have more allergic disorders (15), more autoimmune disease (e.g. Type 1 diabetes and multiple sclerosis), (16–18) and more inflammatory bowel disease (IBD) (ulcerative colitis and Crohn's disease) (19, 20). These tend to rise in parallel in the same countries (18, 21). The increases are real, and are not accounted for by changing diagnostic efficiency. The current view is that these disorders, whether mediated by Th1 (Crohn's disease, Type 1 diabetes, or multiple sclerosis) or Th2 effectors (allergic disorders), are characterized by immune responses to 'forbidden' targets. It is the role of regulatory T cells to stop such unwanted responses, and an imbalance between regulatory T cells (T_{reg}) and $T_{effector}$ cells has now been documented in all these diseases. Thus there is faulty T_{reg} activity in allergies (22), multiple sclerosis (23), autoimmune polyglandular syndromes (24), Th1-mediated cow's milk intolerance (25), and food allergy (26). It is likely to be true for IBD too, though more difficult to prove (27–30). Interestingly, genetic defects in Foxp3, a transcription factor involved in the function of some types of T_{reg} , are associated with a severe illness that includes allergy, autoimmunity, and inflammation of the gut (31).

So why is T_{reg} function deficient in the rich Northern countries? An important part of the explanation is the fact that certain micro-organisms that are part of our evolutionary history are recognized by the innate immune system as harmless, or as organisms that must be tolerated ('Old Friends'), so rather than priming aggressive immune responses they prime immunoregulation (32). For instance, helminths such as *Microfilariae* must be tolerated or elephantiasis will result. Organisms shown to have these properties include certain lactobacilli (33), bacillus species (34), helminths (35), and saprophytic mycobacteria (36). The innate immune system reacts to these organisms by causing a pattern of maturation of dendritic cells (DC) such that these retain the ability to drive T_{reg} rather than effector responses (33, 37, 38). Then, continuing throughput of the 'Old Friends' causes continuous background activation of the DC_{reg} and of T_{reg} specific for the 'Old Friends' themselves, causing bystander suppression. Secondly, the increased number of DC_{reg} that inevitably sample self, gut contents, and allergens, leads to the induction of T_{reg} specific for the target antigens involved in these chronic inflammatory disorders (39). A dramatic example of this phenomenon was seen recently in a five-year study in Argentina of patients with multiple sclerosis (40). Those patients who developed helminth infections (these were not treated) had no further deterioration, and no detectable active lesions. Moreover, they developed myelin-specific T_{reg} that secreted IL-10 and/or TGF- β (40).

The irony is that the recognition of the immunological role of micro-organisms that are part of mankind's evolutionary history, and that are still universally present in large quantities in developing countries, has led to the founding of several biotech companies, and to many clinical trials in the rich countries seeking

to exploit saprophytic mycobacteria (41), helminths (*Trichuris suis*) (42, 43), lactobacilli (44), and bacillus species (34) as therapies for the chronic inflammatory disorders that are becoming so prevalent in rich societies. By contrast, there has been little attempt to apply the understanding gained to the far more significant problems of vaccine design for developing countries. It is, however, clear that a vaccine will tend to induce a greater T_{reg} response in developing countries than in the UK or USA, and this might contribute to its failure.

The Immunology of TB in Developing Countries

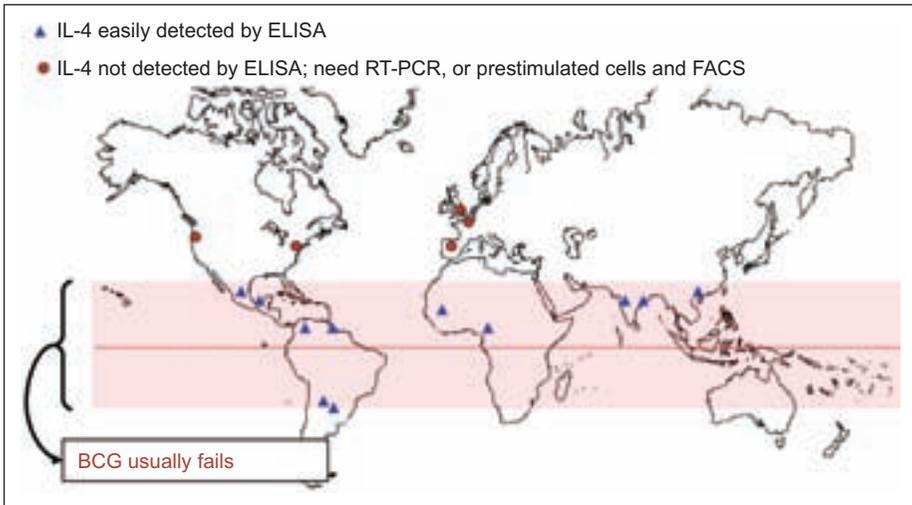
In view of the background priming of Th1, Th2, and T_{reg} responses to mycobacteria by the environment in developing countries, we should anticipate that the immunology of TB will be somewhat different in these areas, which are also the areas where BCG fails.

Interleukin-4

The most obvious characteristic of TB in rich and developing countries is the frequency of patients with high levels of IL-4. We pointed out previously that expression of IL-4 is increased in some TB patients even in rich Northern countries, both in the blood (45, 46) and in the lungs (46, 47). Interestingly, the half-life of mRNA encoding IL-4 is longer in the blood lymphocytes from TB patients than from matched controls (48). However, TB accompanied by very high levels of IL-4 is characteristic of the areas within 30° latitude of the equator, where BCG fails (Figure 28.2) (reviewed in 49). 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 (50). This represents truly enormous levels that are not seen even in a Th2-mediated disorder such as atopic asthma in Europe. In agreement with these findings, an SNP of the Th2-polarizing chemokine CCL2, that causes overproduction of this mediator, is the strongest genetic link with susceptibility to TB in both Mexico and Korea (51). The same SNP is associated with increased severity of atopic asthma symptoms in a European population (52).

Transforming Growth Factor- β

TGF- β levels would be expected to be high in TB in developing countries both because of the priming of regulatory cells already described, and because of secondary induction by IL-4, though it is not yet clear whether levels of TGF- β are also higher in TB in developing countries than in developed ones. When both were measured in a place where IL-4 levels are high, cells from patients with the most advanced TB showed the highest release of both IL-4 and TGF- β (50). Similarly, serum levels of TGF- β were most strikingly raised in patients with advanced disease (53), and a rise was particularly characteristic of MDR patients in Brazil (54).

Figure 28.2 The Geographical Location of TB Patients with Very High Levels of IL-4

Note: Each symbol represents a published study, mostly reviewed in reference 49. Expression of IL-4 is increased in a percentage of patients in all countries, but very high levels, often measurable in serum by ELISA, are characteristic of cavitary TB in countries close to the equator. Note that the failure of BCG close to the equator is due to conditions in developing countries, and is not directly due to latitude.

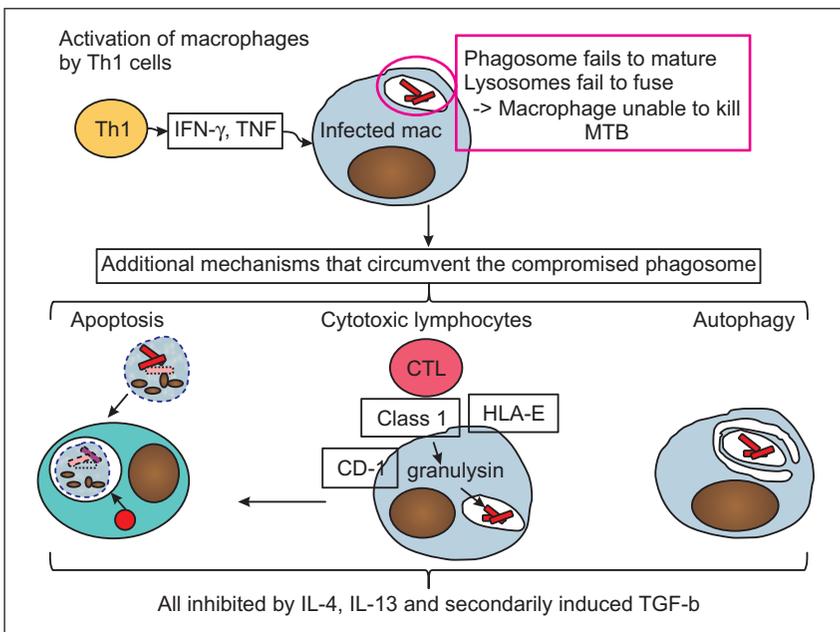
There are strong links between IL-4 and TGF- β . Th2 cytokines, including IL-4 and IL-13, enhance release and activation of TGF- β (55). Remarkably, when expression of TGF- β was followed throughout the course of the infection in a mouse model of pulmonary TB it was found that whereas in normal mice TGF- β expression rose to high levels by day 21 and stayed high thereafter, in IL-4 $^{-/-}$ mice there was an early peak at 7–14 days, followed by barely detectable expression of TGF- β from day 21 until day 120 when the experiment was terminated (56). Therefore, in the context of TB, as in asthma, IL-4 is a major controller of expression of TGF- β , and it will be interesting to see whether TGF- β is disproportionately raised in developing country TB, in parallel with IL-4.

Excessive release of TGF- β has also been implicated in the pathogenesis of TB (50, 57), and it was abundant in tuberculous lung lesions (57). The ManLAM of MTB is a potent inducer of TGF- β (58). Interestingly, human blood monocytes and alveolar macrophages produced bioactive TGF- β upon stimulation by MTB, so a signal from the organism was causing *activation* of TGF- β , as well as its secretion (59). Bronchoalveolar lavage cells from TB patients showed spontaneous and simultaneous production of TGF- β and of the receptors TGF- β R1 and TGF- β R2, required for its activity (60). Natural inhibitors of TGF- β restored T cell responses of patients' cells *in vitro* and augmented monocyte effector function against MTB (61).

The Detrimental Roles of IL-4, other Th2 Cytokines, and TGF- β in TB

There are good reasons why this combination of high Th2 cytokines and TGF- β would be expected to facilitate a secondary phase of bacterial proliferation (62, 63). MTB inhibits classical Th1-mediated macrophage activation because it blocks phagosome maturation (64), lysosome fusion (65), presentation to Th1 cells (66), and triggering via the IFN- γ receptor (Figure 28.3) (67). IL-4 together with IL-13 further impair macrophage function by inducing alternative macrophage activation (68). This involves numerous inappropriate changes, including enhanced iron uptake (69), and down-regulated TLR-2 and TNF, accompanied by increased soluble TNF receptors, DC-SIGN, and IL-10 (70, 71, reviewed in 72). Similarly, TGF- β increases apoptosis of T cells activated by mycobacterial antigens (73–75), and TGF- β enhances growth of MTB in macrophages (76), though not very effectively (77).

Figure 28.3 A Summary of the Mechanisms that Protect from TB



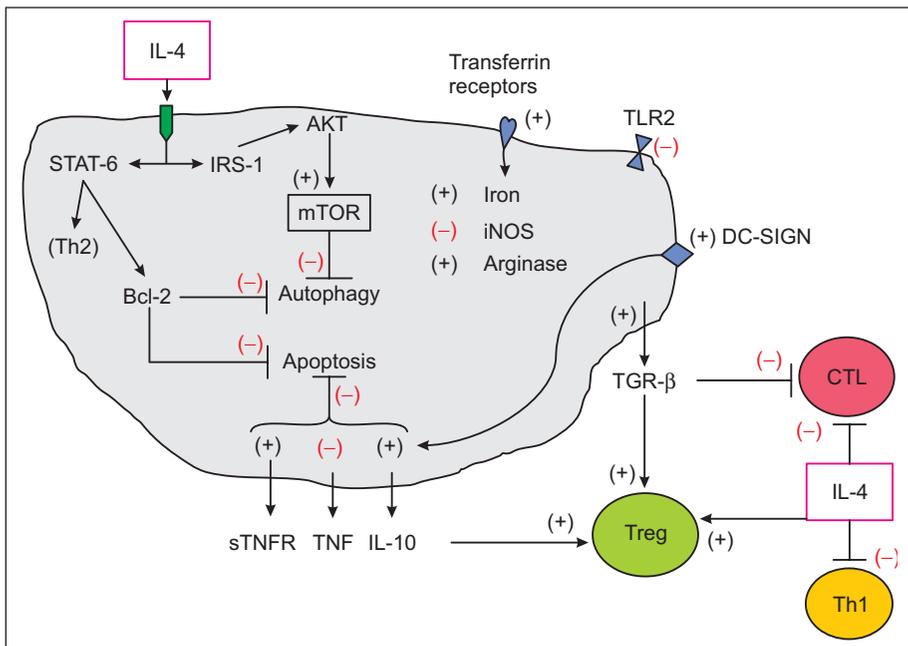
Note: MTB inhibits the classical pathway of cell-mediated immunity; presentation to CD4 cells by MHC Class I, signalling via IFN- γ R, maturation of the phagosome, and lysosome fusion are all blocked. The immune system circumvents the problem of the incapacitated phagosome by means of apoptosis, cytotoxic lymphocytes, and autophagy. Each of these can result in transfer of the organisms to a new fully functional phagosome, or to their exposure to microbicidal peptides.

In the progressive phase of the disease, the immune system depends on several mechanisms that circumvent the failure of the infected phagosome, by promoting its uptake by a fresh macrophage, or directly exposing the bacteria to bactericidal peptides. These mechanisms are illustrated in Figure 28.3, and include apoptosis, CTLs, and autophagy. All of these mechanisms are susceptible to down-regulation by a combination of Th2 cytokines and TGF- β (Figure 28.4) (78–80). Indeed the ability of IL-4 plus TGF- β to impair development and function of cytotoxic lymphocytes is now a major topic in tumour immunology, because tumour-infiltrating lymphocytes can be isolated from the tumour, rendered non-susceptible to TGF- β , and returned to the host, when they can home in to the tumour, and destroy it (79–81).

Pulmonary Fibrosis

Fibrosis is a major complication of TB, which is easily the most important cause of pulmonary fibrosis globally. This might seem odd because IFN- γ opposes fibrosis. Indeed, in humans pulmonary fibrosis tends to be associated with Th2 responses (55). This is because Th2 cytokines, including IL-4 and IL-13, enhance release and activation of TGF- β and, interestingly, when pulmonary TB is established

Figure 28.4 IL-4 Together with IL-13 and TGF- β , Can Inhibit the Rescue Pathways Described in Figure 28.3



Note: Details are outlined in the main text.

in IL-4^{-/-} mice there was markedly less TGF- β , and less fibrosis (56), whereas fibrosis was increased in tuberculous mice with enhanced Th2 activity (82).

Tissue Damage, IL-4, and the Toxicity of TNF

TNF- α is essential for protection (83), but it is clearly toxic in patients with progressive TB, in whom symptoms are alleviated by reducing TNF- α levels (84). In the BALB/c mouse we have shown that toxicity of TNF- α during progressive pulmonary TB is dependent upon the presence of IL-4, and is absent from IL-4^{-/-} mice (56). Toxicity of TNF- α was readily restored in tuberculous IL-4^{-/-} mice by administering recombinant IL-4 (56). The involvement of IL-4 in the toxicity of TNF- α in inflammatory lesions dominated by Th1 cells has been observed in other infectious disease models (85–87). There are a number of possible contributory mechanisms. First, IL-4 causes reduced apoptosis as discussed above, and there is therefore likely to be a corresponding increase in necrosis; apoptotic cells are cleared without inflammation, but necrotic cells trigger further inflammatory reactions. Secondly, there is a large synergy between TNF- α and IL-4 in the induction of expression of VCAM-1 on endothelial cells (88, 89). This synergy results from a combination of transcriptional activation by TNF- α and the stabilization of resulting transcripts by IL-4 (89). Increased VCAM-1 will mediate increased cellular infiltration, and there is evidence that most of the cells present in TB lesions are irrelevant to immunity, and apparently involved in immunopathology rather than protection (90). Thirdly, when T cells are exposed simultaneously to IL-2 and to Th2 cytokines, they become resistant to suppression by glucocorticoid feedback because they express the β splice variant of the glucocorticoid receptor which does not bind glucocorticoids, but antagonizes the transactivating activity of the classic receptor (91). Thus the usual negative feedback on inflammation is diminished, despite enhanced conversion of inactive cortisone to cortisol in the lesions of TB (92). These observations and mechanisms might explain why IL-4 is clearly associated with the extent of pulmonary cavitation (47, 93, 94).

Animal Models After Low Dose and High Dose Challenge with MTB

Another reason why TB with high IL-4 is more prevalent in developing countries emerges from an analysis of animal models. There are two entirely different types of animal models that are accompanied by different immunology, and illustrate the differing roles of Th2 cytokines and TGF- β .

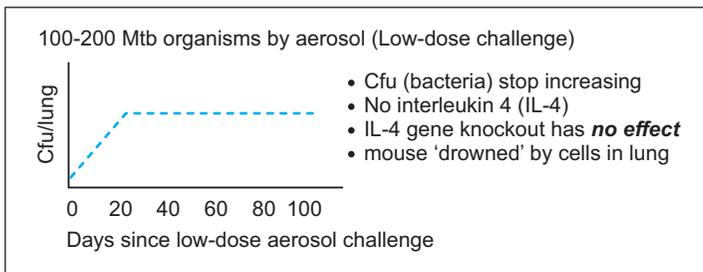
When specific pathogen-free mice are infected by aerosol with 100–200 living MTB, the organisms proliferate for about 3 weeks until a Th1 response

develops (IFN- γ and TNF) (95). Then the proliferation ceases and the viable bacilli in the lungs reach 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 28.5). This probably does not represent a good model of the human disease. The mice die because they are very small animals, and the expanding granulomas rapidly reduce the lung tissue available for respiratory function, despite the effective control of bacterial growth.

This differs from what happens when mice are infected with a high challenge dose of MTB (Figure 28.6). When more than 10^5 MTB are given, either directly into the airways (intratracheal injection) (56, 96) or by intravenous injection (97, 98), a similar plateau is reached at 3 weeks, but after a further 10–14 days bacterial proliferation starts again, and in addition to granulomas there is widespread pneumonia containing MTB. It is the extent of this pneumonia, rather than of granuloma, that correlates with death.

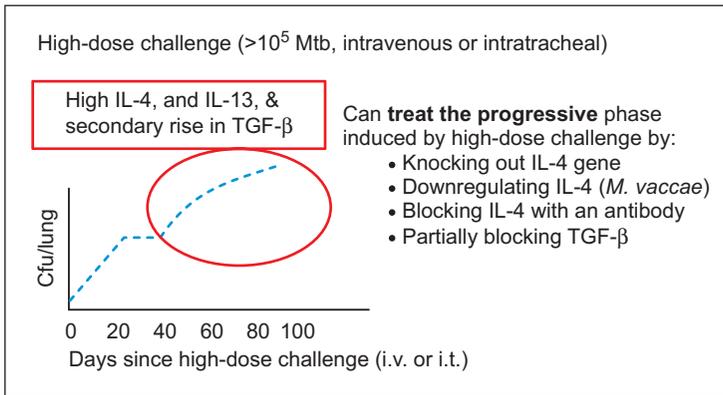
Importantly, this secondary growth of bacilli in the high-dose challenge models is preceded by the appearance of lymphocytes expressing IL-4. However, the role of this IL-4 has been controversial for two reasons. First, high-dose challenge with mycobacteria is known to push the response towards Th2 (99, 100), but that does not prove that the IL-4 is involved in the pathogenesis of TB. Secondly, IL-4 is not involved in the pathogenesis of the low-dose challenge model (95). Mice lacking functional IL-4 genes or STAT-6 are no less susceptible to low-dose aerosol challenge. Nevertheless, it is now proven that IL-4 is directly involved after high-dose challenge. In high-dose models the infection is attenuated in mice with non-functional IL-4 genes (IL-4^{-/-}) (56), and it can be partially treated by administering neutralizing antibody to IL-4 (98). Interestingly, in wild type mice the high-dose infection is accompanied by a marked increase in levels of TGF- β (56). However, in IL-4^{-/-} mice there is a

Figure 28.5 Mouse Models of TB that Use Low Challenge Doses, in Specific Pathogen-Free Laboratory Mice



Note: The proliferation of organisms ceases when the Th1 response develops at about three weeks, but the mice eventually die by drowning in expanding granulomas and cellular infiltration. This is inevitable in such a small animal. Knocking out IL-4 or STAT-6 has no effect on the progress of the disease in these models.

Figure 28.6 Mouse Models that Use High Challenge Doses ($>10^5$) of MTB, Whether Intravenous or Intratracheal



Note: In sharp contrast to low-dose challenge (Figure 28.5), in these high-dose models there is a secondary phase of bacterial proliferation, accompanied by raised IL-4 and TGF- β . These cytokines can be shown to be directly involved in this secondary phase of proliferation, because neutralizing them causes it to diminish or cease, so that the situation returns to one more like that seen in Figure 28.5. This secondary phase of bacillary replication might be the murine equivalent of progressive TB in humans in developing countries, where raised IL-4 and TGF- β are prominent.

brief early peak of TGF- β , which then falls to very low levels (56). This raised the possibility that neutralizing the TGF- β would also treat the disease after high-dose challenge (101). Administering recombinant soluble Type III TGF- β receptors attenuated the disease, and reduced the expression of IL-4 (101). Thus these two cytokines are interdependent in this model, and neutralizing either TGF- β or IL-4 down-regulates the other. Interestingly, neutralizing IL-4 or TGF- β does not cure the animals, but reduces the bacillary load to a plateau, resembling the disease after low-dose challenge (Figure 28.6).

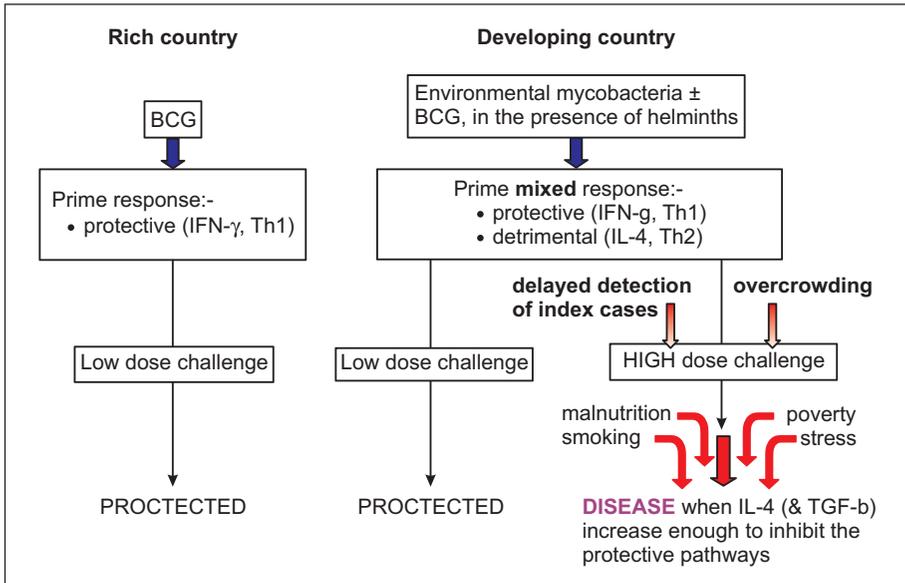
These findings reveal two important facts.

- 1) A high-dose challenge induces a pattern of disease in which IL-4 and TGF- β are increased, even when there is no pre-induced Th2 response.
- 2) In these models the IL-4 and TGF- β play a crucial role in enabling the continuing proliferation of MTB.

Other Causes of Excessive Th2 Activity in Tuberculosis in Developing Countries

Citizens of developing countries are often exposed to high-dose challenge, and so are perhaps analogous to the high-dose challenge animal models discussed

Figure 28.7 Most TB in Developing Countries is due to High-Dose Challenge in Partially Immune Individual



Note: It is suggested that disease occurs, despite partial immunity derived from BCG and environmental mycobacteria, because high-dose challenge, superimposed on a pre-existing Th2 response primed by the environment, can drive enough IL-4, IL-13, and TGF- β to overcome the protective mechanisms. High-dose challenge results from overcrowding, and from delayed diagnosis and treatment of TB cases amongst family members sharing the same room. Malnutrition, stress, and smoking all contribute to activation of the Th2 arm of the response. Vaccination against this type of transmission of TB might be very difficult.

above. However, this factor will be exacerbated by the pre-existing priming of Th2 responses already discussed, and by malnutrition and stress, all of which enhance Th2 responses (Figure 28.7).

High-Dose Challenge due to Overcrowding and Delayed Diagnosis

Since high-dose challenge leads to a form of TB in which IL-4 (and secondarily induced TGF- β) play a significant role, it is logical to ask whether the 'high-IL-4' TB seen in humans in developing countries is also due not only to the pre-existing priming of Th2 responses described earlier, but also to high-dose challenge. In fact there are very strong reasons for believing this to be the case. Overcrowding and delayed treatment of open TB cases in poor countries will inevitably lead to prolonged exposure of family members sharing the same hut or room. High-dose challenge has been shown experimentally to overcome the partial protection provided by environmental mycobacteria (102).

Very brief exposure to a CFU-exPELLING case of TB in Europe or the USA can be sufficient to cause latent infection in some individuals, detected as release of IFN- γ in response to ESAT-6/CFP-10. Thus 15 of 47 adults exposed over 4 days to a case of open TB in an Italian maternity unit were ELISPOT positive 11 weeks later, whereas only 4 were skin-test positive (103). ELISPOT positivity correlated with hours of exposure to the patient, but exposures were inevitably short. Such brief exposures can also lead to progressive disease in certain very susceptible people. For instance, progressive TB occurred following only three 15-minute exposures to an index case (104). Work colleagues of the same patients showed 50 per cent conversion to skin-test positivity, but no disease (104). We can conclude that some humans in Europe and the USA can indeed be 'infected' by very low doses of MTB, leading most often to ELISPOT positivity, more rarely to TST positivity, and very rarely to progressive disease.

However, the situation is different in developing countries. For example, in The Gambia many individuals fail to become even latently infected despite prolonged close contact with open cases of TB (105, 106). This was demonstrated with the ELISPOT assay. Only 38 per cent of those sleeping in the same room as the patient had PBMC that released IFN- γ *in vitro* in response to the relatively MTB-specific antigens, CFP10 and ESAT6, and this was not significantly affected by the presence or absence of a BCG scar (107). There was indeed evidence that proximity (sleeping in the same room, and spending the day in the same compound) and prolonged exposure (> 3 months) increased the likelihood that the ELISPOT test would become positive, but the cumulative numbers of bacilli inhaled before this happened must have been high.

Protein Malnutrition

Protein malnutrition also increases susceptibility, and causes an increase in Th2 activity. Protein malnutrition caused mice to die rapidly from 10^4 MTB *iv*, whereas well-nourished mice survived 10^6 *iv*. When protein was added back to the diet the resistance of the protein-deficient animals rapidly returned to normal (108). Interestingly, CD4+ and CD8+ cells from malnourished children showed increased production of IL-4 and IL-10 and decreased production of IL-2 and IFN- γ compared to cells from well-nourished children (109).

Stress and Poverty

It has been known for many years that psychological stress causes a shift towards a Th2 cytokine pattern. This is partly attributable to glucocorticoids (110, 111), but is also associated with raised levels of various neuropeptides (112). The link between psychological stress and raised serum IL-4 has recently been confirmed in a large scale human epidemiological study (113).

Conclusion

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 (4). This immunity might be able to protect them from low dose challenge, and we suggest that this is how BCG succeeds in rich Northern countries. Part of the apparent failure of BCG in developing countries is in fact a statistical artefact due to the increasing protection derived from contact with environmental mycobacteria that narrows the gap between the incidences of TB in the vaccinated and non-vaccinated populations. However, by the age of 15–20 years essentially everyone in developing countries has this background response, so the disease is occurring in this ‘protected’ group. Why? We suggest that protection fails under the conditions that prevail in developing countries (Figure 28.6): (i) high-dose challenge due to overcrowding; (ii) a pre-existing mixed Th1 and Th2 response to mycobacteria; (iii) the psychological stresses of poverty; and (iv) smoking. Under these conditions, 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 to overcome the protective response, resulting in the characteristic ‘high IL-4’ TB seen in many developing countries (49, 50).

There are major implications. First, models used to select vaccine candidates might need to involve high-dose challenge of partially immune animals in order to mimic the situation in the countries where a new vaccine is most needed. Secondly, it might be useful to document the two immune response patterns (i.e. low IL-4 TB versus high IL-4 TB) in the clinical trial areas, and in particular in trial participants who get disease, because there is a risk that it might be very difficult to vaccinate against TB resulting from high-dose challenge in partially immune individuals.

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