CHAPTER 5.14

THE “OLD FRIENDS” HYPOTHESIS AND TB: IMPACT ON THE DEVELOPMENT OF NEW VACCINES

Graham A.W. Rook

‘Equity happens only when equity is an explicit policy objective.’

Dr Margaret Chan

‘... in the backstage of progress, integral men were continuing integrally the disintegration of helpless living matter.’

Everything Was Going Away

Jacques Prevert

History for a Saint of My Town

Pedro Pablo Oliva

Oil on canvas; 220 × 183 cm
SUMMARY
The epidemiology of TB, and the protective efficacy of BCG vaccine differ strikingly between rich and developing countries. We need to understand these differences in order to optimize the search for novel vaccines and immunotherapies. There has been intense interest in the differences between immune systems in rich and poor countries, but this has been studied in relation to the ‘Hygiene’ or ‘Old Friends’ hypothesis which seeks to explain changing disease patterns in the rich countries. The knowledge gained is being exploited for the benefit of the rich suffering from autoimmune diseases, allergies or inflammatory bowel disease, but it is rarely used to enhance our understanding of diseases of developing countries such as TB. In this chapter I attempt to reassess our understanding of animal models of TB, the immunology of human TB, and the variable efficacy of BCG against the background of the recent ‘Darwinian’ synthesis of the ‘Old Friends’ hypothesis. How different will vaccines need to be if they are to be effective in developing country environments? How relevant are the specific pathogen-free (SPF) animal models upon which we base many of our decisions?

INTRODUCTION
The striking decline in the prevalence of TB in rich countries is mostly attributable to improved housing, nutritional status and medical services. However the modern urban lifestyle has simultaneously brought with it remarkable increases in a wide range of chronic inflammatory disorders including autoimmune diseases [multiple sclerosis (MS), Type 1 diabetes], allergic disorders (asthma, eczema, hayfever), and inflammatory bowel diseases (IBD; Crohn’s disease and ulcerative colitis). This has worried health authorities in Europe and the USA, and led to many studies of the underlying causes. At least part of the explanation lies in the disappearance from the modern urban environment of microorganisms that co-evolved with mammals, already accompanied early hominids in the Paleolithic and are associated with animals, mud and faeces. These organisms that we refer to as ‘Old Friends’, include
helminths, some viruses and gut microbiota, and are discussed and identified later (1). The ‘Old Friends’ often establish stable carrier states, or are encountered continuously in poor rural environments as ‘pseudocommensals’ from mud and untreated water. The ‘Old Friends’ were not lost during the transition from the hunter-gatherer existence to settled agriculture about 10,000 years ago. This transition might even have resulted in increased exposure to them. However the crucial organisms are lost progressively as populations undergo the transition to the modern urban environment. The evidence suggests that these ‘Old Friends’, because they needed to be tolerated by the immune system, have been entrusted by evolutionary selection with crucial immunoregulatory roles, so that when they suddenly disappear, the set points of the immunoregulatory circuits are inappropriate, and chronic inflammatory disorders emerge in genetically susceptible subpopulations. This understanding is leading to clinical trials of several organisms, particularly helminths and probiotics, in allergic disorders, MS and IBD in efforts to re-establish appropriate levels of immunoregulation. However it should be possible to extrapolate this understanding in the other direction, and to ask oneself how the continuing presence of the ‘Old Friends’ in developing countries will modulate the response to TB and to vaccines. How different will vaccines need to be if they are to be effective in such environments? How relevant are the specific pathogen-free (SPF) animal models upon which we base many of our decisions?

THE ‘OLD FRIENDS’ HYPOTHESIS

The ‘Hygiene Hypothesis’ from which the ‘Old Friends’ hypothesis is derived, was initiated by the observation that in young adults a history of hay fever was inversely related to the number of siblings in their family when they were 11 years old (2). This led to several years of narrow focus on allergies, and on the common infections of childhood, with an underlying view that these infections might regulate the Th1/Th2 balance. However excellent studies have indicated that these childhood infections do not protect from allergies (3), and the Th1/Th2 balance hypothesis had been untenable since 1998 (4), by which time it was clear that there was a simultaneous increase in Th1/Th17-mediated chronic inflammatory diseases (Type 1 diabetes, multiple sclerosis, inflammatory bowel disease) occurring in the same countries as the increases in Th2-mediated allergic disorders (5). This strongly suggested that the problem lay not in Th1/Th2 balance but rather in the balance between immunoregulatory and effector mechanisms (whether Th1, Th17 or Th2). A failure of immunoregulatory mechanisms can indeed lead to simultaneous increases in diverse types of pathology, because genetic defects of Foxp3, a transcription factor...
that plays a crucial role in the development and function of regulatory lymphocytes, leads to the X-linked autoimmunity–allergic dysregulation syndrome (XLAAD) that includes aspects of allergy, autoimmunity and enteropathy (6).

Meanwhile epidemiological and experimental studies have progressively identified organisms that have appropriate immunoregulatory properties, and these turn out to be organisms (viruses, bacteria and helminths), often harmless, that co-evolved with man. These can now be divided into two interacting groups: 1) organisms that form part of the co-evolved human microbiota that are altered by modern diets, living conditions and antibiotics, and 2) infections from the paleolithic era, usually harmless, transmitted by the oro-fecal route very early in life, that have been depleted since the transition to the modern urban industrial environment (2nd Epidemiological transition). Below I discuss the evolutionary significance and mechanisms of the immunoregulatory effects of these organisms.

**EVOLED DEPENDENCE**

This term refers to situations where an organism has become dependent on the presence of a partner through loss of genetic material, and can no longer function without that partner (7). A classical example was seen in the laboratory environment when a strain of *Amoeba discoides* became infected with a bacterium (8). Initially this infection compromised the growth of both species, so it was not a case of mutualism. However, after 5 years neither organism could survive without the other. This indicates genetic changes leading to dependence. For instance, an enzyme that is encoded in the genome of both species might be dropped from the genome of one of them. Access to that gene is now ‘entrusted’ to the other species. This idea is at first somewhat alien to immunologists, but is in fact rather commonplace. For instance, almost all mammals can synthesise vitamin C, but large primates and guinea pigs have lost the relevant pathways. Man and guinea pig are now in a state of evolved dependence on fruit and vegetables; we had the genes in the past, but we do not any more. Thus the ‘Old Friends Hypothesis’ postulates that regulation of the mammalian immune system is in a state of evolved dependence on a range of microorganisms with which we co-evolved. The logic here is that these organisms, often picked up as neonates were carried for life, or crucial components of the microbiota, and so had to be tolerated. For example, the consequences of a failure to downregulate the immune response to microfilariae is elephantiasis. The inappropriate immune response merely damages lymphoid tissue. The consequences of immune responses to gut contents are inflammatory bowel disorders.
COMPENSATORY GENETIC VARIANTS

This concept becomes clearer when the genetics are also considered. In parts of the world where there was a heavy load of organisms causing immunoregulation there has been selection for single nucleotide polymorphisms (SNP) or other variants that partially compensate for the immunoregulation. This is seen for several proinflammatory cytokines (9), and IgE (10). There is also an increased frequency of a truncated form of the serotonin transporter that has a marked pro-inflammatory effect (11). The problem here is clear (Figure 5.14.1). As soon as the immunoregulation-inducing organisms are withdrawn by the modern lifestyle, these genetic variants lead to excessive inflammation, and become risk factors for chronic inflammatory disorders (9–11). This constitutes a second layer of evolved dependence on the continuing presence of the ‘Old Friends’ (Figure 5.14.1).

This is important because work that identifies proximate ‘causes’ for diseases that were rare or nonexistent before industrialization and urbanisation may merely be unraveling a problem that would be irrelevant if the microbial status could be returned to that seen in the paleolithic. For instance gluten-associated enteropathies might be an ‘artefact’ of poorly immunoregulated guts. Similarly the recent claim to have discovered that the ‘cause’ of Crohn’s disease is a genetically determined defect in the homing of neutrophils is difficult to reconcile with the fact that 100 years ago the disease barely existed. It is the recent environmental changes that have caused these phenotypes to become risk factors (12) (Figure 5.14.1).

EPIDEMIOLOGICAL TRANSITIONS

In order to be able to identify the relevant organisms, we need to understand the history of man’s changing microbial exposures. Paleolithic populations carried the organisms that they inherited from their primate ancestors (‘heirloom’ species) including many viruses, and organisms that co-evolved with man, as shown in Figure 5.14.2 (13, 14). In addition, they will have consumed several milligrams of harmless environmental saprophytes every day, since these are ubiquitous in soil and water. We have called these ‘pseudocommensals’ because of their inevitable continuous presence until the modern era. The organisms that have been found to be important for the hygiene hypothesis belong within these categories.
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About 10,000 years ago, the shift to agriculture and husbandry created the First (Neolithic) Epidemiological Transition (Figure 5.14.2) (14). This will have had little effect on exposure to the ‘pseudocommensals’ or to the heirloom species. However the more sedentary lifestyle increased orofecal transmission, and caused prolonged contact with animals. The latter led to adaptation to man of a number of animal viruses.

**Figure 5.14.1** Interaction between genetics and loss of the “Old Friends”. The “Old Friends” had to be tolerated and so co-evolved roles as triggers of immunoregulatory pathways. In areas with very high loads of these and other organisms, particularly helminths, compensatory genetic variants accumulated, to partially restore inflammatory responses. In the absence of the Old Friends, not only is immunoregulation inadequately primed, but also these genetic variants cause excessive inflammation and become risk factors for chronic inflammatory disorders. Genetic variants that were advantageous, and did not cause disease in the past, start to do so in the absence of the Old Friends (referenced in main text). Several aspects of modern life are potentially interacting with the lack of “Old Friends” at the level of immunoregulation; obesity, stress, vitamin D deficiency, triggering of Th17 cells by dioxins.

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Figure 5.14.2 The Epidemiological transitions. The hygiene hypothesis implicates organisms that are thought to have accompanied mammalian and human evolution. This relationship was long enough for the establishment of evolved dependence on these organisms, that must be tolerated, and so have developed roles in the initiation of regulatory pathways. Organisms that evolved during the Neolithic are less likely to be relevant in this context, and the 1st Epidemiological transition did not reduce human contact with organisms associated with animals, faeces and mud. On the other hand the 2nd epidemiological transition has led to gene-environment misfit, as the “Old Friends” from the Paleolithic were progressively removed from the modern environment. These are precisely the organisms that have been implicated by epidemiology in the “Hygiene” or “Old Friends” hypothesis. TB is most common in regions that have not yet fully undergone the 2nd epidemiological transition.
shown in Figure 5.14.2 (13). However the viruses acquired during the Neolithic such as influenza (B & C), smallpox, mumps and measles cannot have become endemic until populations were large enough. This required communities of several hundreds of thousands, which did not occur until the appearance of cities 2-3000 years ago. Since this represents only 100-150 generations, extremely strong selection pressure would have been required for evolved dependence to appear, and this seems unlikely. Moreover most humans did not live in such large groups, and these viruses were, for example, absent from pre-columbian American populations.

In short, there were dramatic changes to man’s microbial environment after the 1st Epidemiological Transition, but this did not result in loss of exposure to the organisms implicated by epidemiology in the hygiene hypothesis because until the modern era more than 97% of the population still lived in rural environments, close to mud, animals and faeces which were the sources of these organisms. The situation did not change until the mid 19th Century when industrialization and urbanization began. Since then some populations have undergone a 2nd Epidemiological Transition in which public health measures and more recently, antibiotics, have resulted in diminished (or delayed) exposure to many of the organisms that were present in earlier eras.

THE CRITICAL ORGANISMS AND THEIR IMMUNOLOGICAL ROLE

These considerations allow prediction of the organisms involved in the ‘Old Friends’ hypothesis. From a Darwinian perspective we would expect the relevant organisms to have been present, inevitably and continuously, from relatively early in the evolution of the immune system (‘Old Friends’). One would also anticipate a reliable mode of transmission such as the orofecal route, often accompanied by the ability to establish carrier states that facilitate such transmission.

Gut flora

Proof, using modern methods, that the microbiota of city-dwelling European (EU) children differs dramatically from that of rural Africans was obtained recently (15). The fecal flora of children from Burkina Faso (BF) had more Bacteroidetes and less Firmicutes and Enterobacteriaceae. Only the BF children had organisms (Prevotella and Xylanibacter) known to contain for hydrolysis of cellulose and xylan which may have contributed to the fact that BF children had more short-chain fatty acids
SCFA (SCFA) than did EU children (15). SCFA have a protective anti-inflammatory role in the gut (16, 17). There was also significantly greater microbial richness and biodiversity in BF samples than in EU samples (15). This is important because a decrease in the abundance and biodiversity of Firmicutes has been observed repeatedly in Crohn’s disease (CD) patients (18). Interestingly, *Faecalibacterium prausnitzii*, an anti-inflammatory commensal bacterium that also contributes to SCFA generation, was present in the microbiota of the BF children (15), but is often lacking in European CD patients (18).

Although this paper is concentrating on chronic inflammatory disorders, obesity is known to be pro-inflammatory, and low levels of Bifidobacteria and high levels of *Staphylococcus aureus* in infant microbiota may predict the development of obesity later in life (19).

The virome of the microbiota is now also being studied. Most of the viruses are bacteriophages but how they vary or affect the immunoregulatory effects of the bacterial microbiota is not yet known (20).

**Orofecoally transmitted chronic infections**

The orofoeally transmitted organisms highlighted in recent studies of the hygiene hypothesis include *Helicobacter pylori*, *Salmonella*, Hepatitis A virus (HAV), enteroviruses and *Toxoplasma gondii* (21–24). Protozoa not yet considered in this context, to my knowledge, include the very ancient *Entamoeba*, *Giardia*, and *Trichomonas* all of which have lost their mitochondria and have very long and close associations with humans.

**Helminths**

The role of helminths is best established for allergic disorders (25) and multiple sclerosis (26, 27). Many helminths are also orofoeally transmitted or rapidly picked up from the environment. Interestingly an intestinal helminth infection can modulate the microbiota, in addition to exerting direct immunomodulatory effects discussed later. Infection with *Helimosomoides polygyrus* caused a significant increase in Lactobacillaceae in the ileum of infected mice (28).
Other categories of ‘Old Friends’

We have discussed elsewhere other categories of organisms that have changed dramatically in the modern western lifestyle. These include skin flora that contribute to nitric oxide cycles by metabolising ammonia and nitrate in sweat (29), organisms in breast milk (30), environmental ‘pseudocommensals’ (31, 32), and ectoparasites that inject immunomodulatory substances while taking blood feeds (33).

Immunoregulatory effects of the ‘Old Friends’

Numerous mechanisms exist, but there is a single underlying evolutionary principle that probably applies to all the ‘Old Friends’. These organisms persist as commensals, carrier states or chronic subclinical infections. Therefore the host-parasite relationship evolved so that rather than provoking needless damaging aggressive immune responses, an anti-inflammatory equilibrium is established. Some mechanisms are summarized in Figure 5.14.3. Many involve dendritic cells (DC).

Dendritic cells

A frequent mechanism is modulation of dendritic cells (DC) such that these drive Treg rather than Th1, Th17 or Th2 effector cells (referenced in 34). Then the constitutive presence of the ‘Old Friends’ causes continuous background activation of the DCreg and of Treg specific for the ‘Old Friends’ themselves, resulting in background bystander suppression of inflammation. Meanwhile these DCreg inevitably sample self, gut contents and allergens, and so induce Treg specific for the illicit target antigens of the three groups of chronic inflammatory disorder. Release of the anti-inflammatory cytokines, IL-10 and TGF-β is often involved in the anti-inflammatory effects of these cells (31). A striking example of this in human autoimmunity is a recent experiment of nature. Patients in Argentina suffering from multiple sclerosis were followed up for 4.6 years. It was found that those who developed parasite infections (which were not treated) had significantly fewer exacerbations than those who did not (35). Moreover, they also developed regulatory lymphocytes that specifically responded to myelin basic protein. In other words, the presence of the parasite appeared to drive the development of regulatory cells that recognised the autoantigen and inhibited the autoimmune disease process. The parasites acted as ‘Treg adjuvants’.
Figure 5.14.3 A few of the mechanisms involved in the immunoregulatory and anti-inflammatory properties of the “Old Friends” and microbiota. A key pathway is the modification of DC so that they tend to drive Treg. Such DC also process self antigens, allergens etc., and so drive crucial specific regulatory cell populations that block autoimmunity, allergies and inflammatory bowel disease. The intestinal helminths also exert indirect effects by modulating the microbiota. The individual pathways (some taken from experimental systems in the mouse, as illustrations) are referenced in the main text. (ESA, excretory-secretory antigen of Heligmosomoides polygyrus. PSA, polysaccharide antigen of Bacteroides fragilis. SCFA, short chain fatty acids. RA, retinoic acid. DC, dendritic cell. Aldh1a2, Retinaldehyde dehydrogenase 2. GPR43, G-protein-coupled receptor 43. RegIIIg, regenerating islet-derived 3γ. CD103, an integrin, and marker of intestinal regulatory DC. IDO, indoleamine 2,3, dioxygenase).
Immunoregulation by helminths

Little is yet known about the precise molecular signals involved in immunoregulation by helminths. For some helminths it seems that complex oligosaccharides are important, especially those that mimic human oligosaccharides. Dendritic cells (DC) express many different C-type lectin receptors on their membranes, which vary within distinct DC subsets. For example DC-SIGN, macrophage galactose-type C-type lectin (MGL, CD301) and the mannose receptor (MR), are all expressed by DCs and shown to interact with host-like glycans of helminths in ways that are thought to provoke immunoregulatory changes that assist parasite persistence (36). *Heligmosomoides polygyrus* excretory-secretory antigen binds the TGF-βRII and causes FoxP3+ T cells to become functional Foxp3+ Treg (37). But this helminth also modulates DC function in an anti-inflammatory way (38), and may exert indirect immunomodulatory effects via induced changes in the bacterial microbiota (28).

Hepatitis A virus

A number of epidemiological studies have demonstrated an association between infection with the hepatitis A virus (HAV) and protection against the development of asthma. Interestingly TIM-1 (T cell, immunoglobulin domain and mucin domain) which is the cellular receptor for the virus is an important atopy susceptibility gene. Furthermore, recent studies indicate that TIM-1 is a receptor for phosphatidylserine, a marker of apoptotic cells (39). It is not yet clear how this translates into effects on immunoregulatory pathways.

Gut flora and immunoregulation

This topic was extensively reviewed recently, and Figure 5.14.3 lists some of the known mechanisms (40, 41). In the 1980s it was revealed that defined alterations to the microbiota could reproducibly either increase or decrease susceptibility to autoimmune arthritis (42). Similar findings have been published recently using experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (43). Modulation of the bowel flora could alter susceptibility to EAE by mechanisms that involved the ability of intestinal DC to prime Th1, Th17 or Treg responses (43). SCFA also have an anti-inflammatory role in the gut. SCFAs bind the G-protein-coupled receptor 43 (GPR43, also known as FFAR2), and exert an anti-inflammatory effect that proved relevant in models of colitis, arthritis and asthma (16). Other mechanisms include induction of regulatory B cells (27), regulatory macrophages (44), modulation of Treg/Th17 balance (45), and indirect effects via epithelial cell products that cause DC to drive Foxp3+ cells with gut-homing
properties (46), and induced secretion of REGIIIγ, a C-type lectin with bactericidal effects on Gram-positive bacteria (47). A recent pioneering study has used microarray technology to prove that oral probiotic *Lactobacillus plantarum* can induce changes in gene expression in normal human duodenum. After consumption of midlog *L. plantarum* there was a lack of induction of immune response-associated genes but instead, induction of genes associated with anti-inflammatory activities (48). Some probiotic strains can cause DC to become anti-inflammatory and to drive development of Treg when transferred *in vivo* (49).

Interestingly there is evidence that very clean Specific Pathogen Free (SPF) mice have abnormally functioning Treg that can fail to secrete IL-10, and can switch function to an aggressive cell type (50). Humans in rich Western cities are not SPF, but some modern babies must be getting close to the SPF state. But in developing countries this is clearly not the case.

**INTERACTIONS WITH OTHER ASPECTS OF MODERN LIFESTYLES**

Decreased exposure to microbial ‘Old Friends’ is not the only reason for the increasing frequency of chronic inflammatory disorders in developed countries. Other aspects of modern life must contribute, and are likely to interact with and amplify the immunoregulatory deficit resulting from the altered microbial environment. For instance, the powerful protection from allergic disorders provided by early exposure to cowsheds (51) might be in part due to exposure to immunomodulatory arabinogalactans derived from grass (52), rather than to microorganisms. Similarly the protective effect of unpasteurized farm milk direct from the cow is not understood (53), but is probably not microbial. Nevertheless, these are clearly factors that differ between TB-rich developing countries and rich urban communities.

Diet and obesity are associated with modified gut flora (54), which will have immunoregulatory consequences (41). Psychological stress also modulates gut flora and gut permeability, while both obesity and stress result in greater release of proinflammatory cytokines (55). Similarly, vitamin D is involved in driving some subsets of regulatory cells (56). Paranoia about the carcinogenic effects of sunlight is causing deficiency of vitamin D to become extremely common, and it is increasingly implicated in the increases in chronic inflammatory disorders. Finally, pollution, particularly dioxins, which drive Th17 cells via the aryl hydrocarbon receptor (57), will also encourage inflammatory responses.
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EXPLOITATION OF THE "OLD FRIENDS" HYPOTHESIS IN RICH COUNTRIES

There are numerous experimental models in which exposure to microorganisms that were ubiquitous during mammalian evolutionary history, but are currently ‘missing’ from the environment in rich countries (or from animal units with Specific Pathogen-Free facilities) will treat allergy (31, 32), autoimmunity (58) or intestinal inflammation (59). As anticipated, the therapeutic effect is attributable to immunoregulation. Animal models using helminths were reviewed recently (60).

Clinical trials in humans using hookworm (*Necator americanus*) or *Trichuris suis* have been completed (59, 61, 62). The focus has been on allergies and inflammatory bowel disease (59, 61, 62). In Brisbane a trial of hookworm has been completed in coeliac disease (http://clinicaltrials.gov/show/nct00671138). Hookworm did not have much impact, if any, on clinicopathological measures of celiac disease in the context of a robust gluten challenge. However, it does seem that hookworm dampens the gluten-specific Th1 and Th17 responses (John Croese, personal communication). Trials in multiple sclerosis are also in progress. Meanwhile interest is growing in the increases in some cancers that are directly attributable to chronic inflammation, or to which inflammation provides growth and angiogenic factors (50, 63). Finally, since chronically raised pro-inflammatory cytokine levels are associated with depression and can cause depression, which is often comorbid with other chronic inflammatory diseases, the potential importance of the ‘Old Friends’ hypothesis in depression has been coming under scrutiny (64). So there is much activity in relation to the problems of the rich countries. But are we applying this knowledge to problems of the developing world such as TB?

THE IMPLICATIONS FOR TB

Many of the organisms implicated in the ‘Old Friends’ hypothesis are also important in our understanding of TB. Moreover, when looked at from the point of view of the poor countries, the ‘Old Friends’ hypothesis suggests that there will be significant differences in immune systems, particularly a tendency to greater immunoregulatory responses. These issues are discussed below.
Partially protective Th1 responses induced by environmental mycobacteria

Saprophytic mycobacteria (constituents of the ‘pseudocommensals’ discussed earlier) are ubiquitous, but the degree of sensitisation 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 immunisation by a range of environmental mycobacteria was much greater in young Malawians (65). Even within the USA sensitisation detected by skin-testing is highest in agricultural workers in humid Southern areas, and lowest in middle class USA-born caucasians in the North (66). In humid developing countries more than 90% of the population has a positive skin-test response to environmental mycobacteria by the age of 15 to 20 years (67). There is good evidence that this sensitisation has some protective effect against MTB (68). 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 5.14.4).

Overcrowding and challenge dose

Since exposure to environmental mycobacteria has a protective effect, why is TB so prevalent in developing countries? One factor is exposure to high dose challenge. This has been shown experimentally to overcome the partial protection provided by environmental mycobacteria (69).

In Europe or the USA very brief exposure to a CFU-expelling case of tuberculosis can be sufficient to cause latent infection in some individuals, detected as release of IFN-γ in response to ESAT-6/CFP-10 (70). 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 tuberculosis occurred following only three 15 minute exposures to an index case (71). Work colleagues of the same patients showed 50% conversion to skin test positivity, but no disease (71). 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 tuberculin skin-test 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 (72, 73). This was demonstrated with the ELISPOT assay. Only 38% of those sleeping in the same room as the patient had peripheral blood mononuclear cells that released IFN-γ \textit{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 (74). 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. In crowded living accommodation, especially when TB cases go undiagnosed, exposure to MTB may occur over many months, leading to high challenge doses (Figure 5.14.5). It is interesting that recent

\textbf{Figure 5.14.4} Masking the protective effect of BCG vaccination by protective environmental mycobacteria. Part of the apparent failure of BCG in developing countries is an artefact due to the increasing protection derived from contact with environmental mycobacteria. With time this narrows the gap between the incidences of TB in the vaccinated and non-vaccinated populations. In fact both are partially protective, but fail to protect adequately against the repeated high dose challenges that occur in crowded developing country conditions.
work has shown that many of the organisms in sputum are in a dormant state (75), so a steady accumulation of bacteria may result, until progressive disease eventually occurs. As discussed in detail in 2C.3 of this volume, high challenge doses tend to drive the response towards Th2 (Figure 5.14.5).

**Figure 5.14.5** The relevance of dose of challenge. A subset of individuals develops progressive TB after a single low dose challenge, though most do not. BCG might protect against such challenges. However in developing countries partial immunity due to BCG and to environmental mycobacteria usually protects against low dose challenge, but frequently fails when challenge doses are high and repetitive, as will occur in crowded poor-quality accommodation. This failure is aggravated by factors that enhance Th2 responses, such as stress, poverty, malnutrition and smoking. It is likely that the immunology of the two types of TB is different, and that the genetic factors leading to susceptibility to progressive disease following sporadic low dose exposure in the USA are different from the genetic factors involved in progressive disease following high dose challenge of partially immune people in a developing country.
The tendency of high challenge doses to overcome immunity and bias the response to Th2 is increased by protein malnutrition, smoking and psychological stress (Figure 5.14.5). Protein malnutrition caused mice to die rapidly from $10^4$ *M. tuberculosis* i.v., whereas well-nourished mice survived $10^6$ i.v. When protein was added back to the diet the resistance of the protein-deficient animals rapidly returned to normal (76). 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 (77). Similarly, it has been known for many years that psychological stress causes a shift towards a Th2 cytokine pattern. This is partly attributable to glucocorticoids (78, 79), but is also associated with raised levels of various neuropeptides (80). The link between psychological stress and raised serum IL-4 has recently been confirmed in a large scale human epidemiological study (81).

**Priming of Th2 responses to mycobacteria in developing countries**

This tendency for developing country living conditions to drive the response towards Th2 occurs in a situation where a Th2 component to the response to mycobacteria has already been primed in most people in childhood (65, 82, 83), possibly because of the simultaneous presence of helminths. For instance when PBMC from children in Sanaga valley of Cameroon were cultured for 2 days with PPD, IL-4 output increased linearly with increasing age up to 16 when it reached 50-60 pg/ml (82). In brief, the presence of an obvious IL-4 response to TB is particularly notable in the developing countries where BCG fails, and the Old Friends are still ubiquitous (reviewed in 84). Interestingly TB patients have IgE antibody to MTB itself (85). This striking point is often forgotten, as is the presence of equally IL-4-dependent IgG4 antibody (86, 87), suggesting that the Th2 component becomes exaggerated during active disease.

**The role of helminth infections**

It is reasonable to implicate some helminths in the induction of Th2 and of 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 (88). Second, total IgE and Ascaris-specific IgE levels were high in TB patients, and total IgE declined following successful treatment of TB (89, 90).
In Brazil, a higher prevalence of intestinal nematodes was seen in patients with TB compared to a matched control group: 57.8% versus 20.9% (91). In a study in Ethiopia, the most prevalent helminth infection was *Ascaris lumbricoides*. This was present in 53.5% of TB patients, but only 19.8% of controls selected from the same households, so poverty and overcrowding should not be confounders (92).

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 (93). This progression was also associated with IL-5 and IL-10 responses to mycobacterial antigen (94). 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 (95). 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 (95).

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 ‘Old Friends’ hypothesis predicts that in developing countries where the chronic inflammatory disorders (autoimmunity, allergic disorders and IBD) are relatively rare, the population will have a greater tendency to develop regulatory cell responses to antigens that they encounter. This will include mycobacterial antigens as outlined above. Therefore the background response to mycobacteria will not only be mixed Th1/Th2 but also heavily regulatory as well (96, 97). Comparative studies will be required to check whether this is more striking than in European patients (98).

**ANIMAL MODELS OF TB**

Many of these points are reflected in a comparison of animal models of TB in rich and developing countries. When very clean specific pathogen-free (SPF) mice in Europe or the USA are infected by aerosol with 100-200 living MTB. (MTB), the organisms proliferate for about 3 weeks until a Th1 response develops (IFN-γ and TNF) (99). IL-4 is not involved in this model (99). Then the proliferation ceases and
the viable bacilli in the lung reach a plateau. The animals eventually die, but this is due to expanding granulomas and cellular infiltration rather than to proliferation of MTB. The air volume of the lungs of a relatively large mouse is about 300-400 μ. Therefore 200 granulomas will ‘drown’ a mouse before they reach 1.5 mm in diameter (total of 353.4 μ, assuming that they are spherical), whereas these would not be noticed in a human. These low dose infection models, where bacterial proliferation ceases, are models of latent TB, not of progressive disease.

By contrast, in some laboratories bacterial replication restarts after the Th1 response has developed. This is most often because a larger challenge dose has been used. If more than $10^5$ MTB are given, either directly into the airways (intratracheal) (100, 101) or by intravenous injection (102, 103), a similar plateau may be detectable at 3 weeks, but this is transient, and a few days later bacterial proliferation starts again. This phase of progressive disease depends on IL-4 and TGF-β, and partial treatment can be achieved by knocking out (100) or blocking these mediators (103, 104). Interestingly, in Mexico (105) or Brazil (101) it is essential to give this larger challenge dose, because 100-200 organisms can fail to cause disease. It has recently emerged that the breeding pairs in both Brazil (106) and Mexico (Y. Lopez Vidal and R. Hernandez Pando, personal communication) are exposed to environmental mycobacteria. Thus, this resistance to low dose infection parallels that seen in human populations in the same developing country environments (107), and is due to prior contact with mycobacteria. Again, high dose challenge helps to bias the response towards Th2, which blocks the protective response as described in detail in Chapter 2C.3 and allows true progressive disease to develop.

**CONCLUSIONS**

These ways in which immune systems differ in developing countries have many direct implications for the management of TB. It is often stated that BCG fails to protect from TB in developing countries. This assertion needs to be qualified. We know that environmental mycobacteria can exert a protective effect, and a recent reappraisal of the South India BCG trial has shown that BCG did protect those individuals who were mycobacterially naïve according to skin tests at the time of vaccination. This protection was clear when they were compared to individuals who were also mycobacterially naïve, but did not receive BCG (68, 108). The degree of protection provided by BCG was slightly less than that derived from environmental mycobacteria, and so was masked unless the mycobacterially naïve individuals were considered separately. The problem then, in developing countries,
is not that BCG does not do the same as in rich developed countries, but that this is not sufficient to protect from high dose challenge in undernourished stressed individuals, where the response can be diverted towards Th2. So perhaps the ability to block deviation towards Th2 is the property that we should be seeking when screening vaccines?

However, this is not what has been happening. The mouse models that have been used as the first screening step in the search for potential vaccines seem closer to human latent disease than to progressive disease. Thus it is possible that we have chosen candidates that accelerate arrival of the Th1-mediated plateau, or lower the CFU at which the plateau occurs, rather than vaccines that can oppose the deviation to Th2 and excessive TGF-β that occurs following high dose challenge. This could be rectified easily by testing vaccines in models that use high dose challenge. Similarly, if helminths, with their potent Th2-biasing and Treg-adjuvant effects are important, then we should be using high dose challenge models with helminth co-infection. For instance, it might be useful to evoke Treg (or other regulatory cell types) with the ability to inhibit Th2 responses, but not Treg that inhibit Th1 responses (109).

Immunotherapy is also potentially important. In the progressive phase of the high dose challenge models, several strategies are able to exert significant therapeutic effects in the complete absence of chemotherapy. These include anti-IL-4 (103), partial inhibition of TGF-β (104) and high doses human Intravenous immunoglobulin (110). All of these could go into clinical trial, perhaps to shorten chemotherapy, or as rescue therapy for untreatable cases of extensively drug-resistant TB (XDR-TB).

Finally, the views expressed here suggest that there must be two broad overlapping categories of Tb patients. First, there are those who, presumably for genetic reasons, are susceptible to progressive disease following low dose challenge. Secondly there are also individuals who get progressive disease only after high dose challenge. This can occur despite pre-existing partial immunity, if that immunity becomes diverted towards IL-4 and TGF-β. The immunology and genetics of these two populations will be entirely different, and this might explain some of the variation in findings and genetic associations in different studies.
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THE "OLD FRIENDS" HYPOTHESIS AND TB:
IMPACT ON THE DEVELOPMENT OF NEW VACCINES

CHAPTER 5.14

