Interaction of Mycobacterium tuberculosis with the host: consequence For Vaccine development

Article *in* Apmis · June 2009

Impact Factor: 2.04 · DOI: 10.1111/j.1600-0463.2009.02458.x · Source: PubMed

2 authors:

Jes Dietrich
Statens Serum Institut
80 PUBLICATIONS **3,360** CITATIONS
SEE PROFILE

Timothy Mark Doherty
GlaxoSmithKline plc.
160 PUBLICATIONS **6,372** CITATIONS
SEE PROFILE

APMIS 117: 440–457

DOI 10.1111/j.1600-0463.2009.02458.x

Interaction of *Mycobacterium tuberculosis* with the host: consequences for vaccine development

JES DIETRICH and T. MARK DOHERTY

Department of Infectious Disease Immunology, Statens Serum Institute, Copenhagen, Denmark

Dietrich J, Doherty TM. Interaction of *Mycobacterium tuberculosis* with the host: consequences for vaccine development. APMIS 2009; 117: 440–57.

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), remains a major worldwide health problem that causes more than 2 million deaths annually. In addition, an estimated 2 billion people are latently infected with *M. tuberculosis*. The bacterium is one of the oldest human pathogens and has evolved complex strategies for survival. Therefore, to be successful in the high endemic regions, any future TB vaccine strategy will have to be tailored in accordance with the resulting complexity of the TB infection and anti-mycobacterial immune response. In this review, we will discuss what is presently known about the interaction of *M. tuberculosis* with the immune system, and how this knowledge is used in new and more advanced vaccine strategies.

Key words: Tuberculosis; bacterial; vaccination; BCG; latency.

Jes Dietrich, Department of Infectious Disease Immunology, Statens Serum Institute, Artillerivej 5, DK-2300 Copenhagen, Denmark. e-mail: JDI@ssi.dk

INTRODUCTION

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is one of the world's most devastating human pathogens. In 2004, >9 million people developed active TB and approximately 2 million people died from it, making this disease the second leading cause of infectious disease mortality worldwide (1). Central to the success of *M. tuberculosis* as a pathogen is its ability to persist within humans for long periods in a clinically latent state: roughly 95% of the people who become infected develop a latent infection. The magnitude of this disease reservoir is estimated to be approximately 2 billion people or roughly one-third of the global population (2). The problem is made worse by the interaction of *M. tuberculosis* and HIV and the two infections intersect in the world's poorest countries, magnifying the death toll. As a result, TB is the leading cause of death in HIV-infected individuals. Infection with HIV increases the risk of TB and also increases the risk of reactivating latent disease to over 20 times that in HIV-negative people as immunosuppression worsens (3, 4). *M. tuberculosis* infection also worsens HIV: people living with HIV and active TB tend to have higher viral loads and die sooner than those without TB (5–7). Furthermore, anti-TB drugs, mainly rifampicin, have important interactions with antiretroviral drugs (8), while HIV treatment in people coinfected with mycobacteria can lead to the potentially fatal immune reconstitution inflammatory syndrome (9, 10). All of this makes TB control a priority issue around the globe.

In this review, we will introduce the disease, and then focus first on the complex interaction of *M. tuberculosis* with the immune system (on a cellular level). Thereafter, we will focus on the interaction with the host. In light of this, we will then discuss the challenges that vaccine developers face.

Invited review

GLOBAL TB CONTROL

TB can be cured in most cases by a cheap course of antibiotic treatment, but the difficulty of a timely diagnosis, socioeconomic factors in TB-endemic areas and the fact that bacterial clearance requires many months of treatment have combined to prevent successful global TB control by antibiotics. In addition, the emergence of multidrug-resistant TB (MDR TB) and extremely drug-resistant TB of (XDR TB) has highlighted the importance of an increased effort against TB. MDR TB is a strain that is resistant to at least two of the best anti-TB drugs, isoniazid and rifampicin, that form the core of standard treatment. XDR TB is still relatively rare [an estimated 5% of cases (1)] but combines resistance to isoniazid and rifampin with resistance to the best second-line medications: fluoroquinolones and at least one of three injectable drugs (i.e., amikacin, kanamycin or capreomycin). Patients with XDR TB are left with treatment options that are much less effective and often have worse outcomes. Thus, it is not uncommon that people with XDR TB die even after entering treatment (11).

Vaccination has also been only partially successful, despite the fact that the only current vaccine against *M. tuberculosis*, *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG), is the most widely used vaccine in the world. While it has clear beneficial effects against TB in childhood (12, 13) it only provides protection against the disease for a limited number of years (14) in highly TB-endemic regions. The time frame for the waning of BCG-induced protection through childhood and young adult life coincides with the gradual increase in TB incidence, which, in some highly TB-endemic regions, such as sub-Saharan Africa, reaches a peak of >500 cases per 100 000 individuals in the 25–35-year-old age group. In addition, it appears that BCG is ineffective in individuals pre-sensitized to mycobacteria, for example, by exposure to environmental mycobacteria, prior BCG vaccination or *M. tuberculosis* infection (15, 16). BCG is a live vaccine and the development of protective immunity after BCG vaccination appears to require BCG replication in the host, which can be prevented by a pre-existing immune response that can cross-react with BCG (17). The failure of BCG in sensitized individuals means that BCG cannot be used as a booster vaccine to counteract the waning effect of the BCG vaccination given after birth – as attested to by the failure of attempts to boost protection by administering multiple doses of BCG (15, 16). On a global scale, widespread latent TB infection in adults is moreover a significant barrier to attempts to boost immunity. Therefore, a new vaccine is urgently needed. However, *M. tuberculosis* is one of the oldest human pathogens and has evolved strategies for survival. Despite the fact that it stimulates a strong immune response by the host (and in fact is dependent on it for continued dispersal), *M. tuberculosis* has evolved to resist the body's attempts to eradicate it. Thus, designing a new, effective vaccine means understanding why natural immunity fails. Therefore, a novel vaccine to replace (or improve) BCG faces not just one, but many daunting technical problems.

IMMUNOPATHOLOGY AND *M. TUBERCULOSIS* INFECTION

M. tuberculosis normally enters the host through the mucosal surfaces – usually via the lung after inhalation of infectious droplets from an infected individual, occasionally via the gut after ingestion of infected material (for example milk – a common route for the TB complex member, *M. bovis*). Either way, the bacteria can be taken up by phagocytic cells that monitor these surfaces, and if not swiftly killed, can invade the host inside these cells. Some heavily *M. tuberculosis*-exposed individuals show no signs of infection: no pathology, no symptoms and no apparent adaptive immune response. It is possible that in these cases, the innate immune response has eliminated the pathogen at the earliest stage (see Fig. 1). More commonly, however, ingestion of the bacteria by an antigen-presenting cell (APC) rapidly induces an inflammatory response. Cytokine and chemokine release triggers the swift accumulation of a variety of immune cells and, with time, the formation of a granuloma, characterized by a relatively small number of infected phagocytes, surrounded by activated monocyte/macrophages and, further out, activated lymphocytes (18). If the infection is successfully contained at this stage, the granuloma shrinks and may

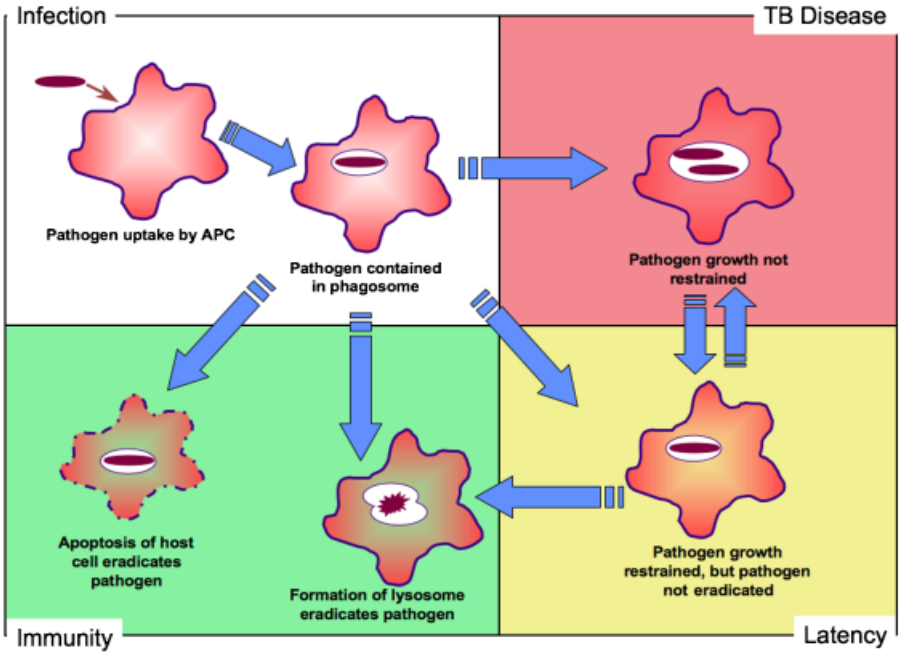

Fig. 1. A simple schematic of the outcomes of *Mycobacterium tuberculosis* infection at the level of the infected host cell – normally a macrophage. If the disease is arrested at the very first stage, an exposure to *M. tuberculosis* may be entirely 'silent' – without symptoms or a detectable specific immune response. If, however, it progresses to any of the other stages – indicated by colored boxes – then *M. tuberculosis* infection becomes overt, with signs ranging from conversion of the tuberculin skin test or positivity in other immune tests, through X-ray changes all the way to full-blown disease. There are two important points to remember, however. Regardless of the outcome at the cellular level, at the level of the host organism, this process is not linear. Patients can – and do – shift between latent and overt disease by reactivating an earlier infection. Likewise, overt tuberculosis disease can be cured – either spontaneously or by chemotherapy – leading to latent disease. There are also data to suggest that latent infections can be eradicated, leading to true immunity.

eventually disappear, leaving a small scar or calcification and the patient's T cells become responsive to *M. tuberculosis*-derived antigens. If, however, the immune response does not successfully control the bacterial replication, the granulomas increase in size and cellularity. Eventually, cell death in the granuloma leads to necrosis. In this case, if the granuloma is close to the surface of the lung, the tissue destruction caused by necrosis can breach the mucosal surface and the granuloma contents leak into the lumen of the lung – a process referred to as cavitation. This gives rise to the prototypic symptom of TB – a persistent cough with blood in the sputum. At this point, the patient is highly infectious, spreading the bacteria by aerosol.

Tissue destruction in TB is not mediated by the activities of the bacteria alone – it is primarily immunopathological in nature and the crucial point to understand is that an inflammatory immune response is critical for the survival of both the host and the bacteria. It thus appears that *M. tuberculosis* actively stimulates – and then subverts – this response. The outer surface of *M. tuberculosis* contains a number of molecules that bind to the host's pathogen-associated molecular pattern (PAMP) receptors, such as the Toll-like Receptor (TLR) family (19). Thus, although engagement of PAMP receptors appears to be a crucial initial step for anti-mycobacterial immune responses (20, 21), all clinical strains of *M. tuberculosis* express a number of molecules (both expressed on the bacteria's surface and secreted) that trigger these pathways. Interestingly, the majority of these molecules do not seem to be crucial to mycobacterial viability and as this pathogen has a long co-evolutionary history with human beings (22, 23), it suggests that their conservation serves another important function. The simplest explanation is that *M. tuberculosis* depends on the immunopathology that promotes cavitation for spread to new hosts. A failure to stimulate inflammatory immune responses is therefore an

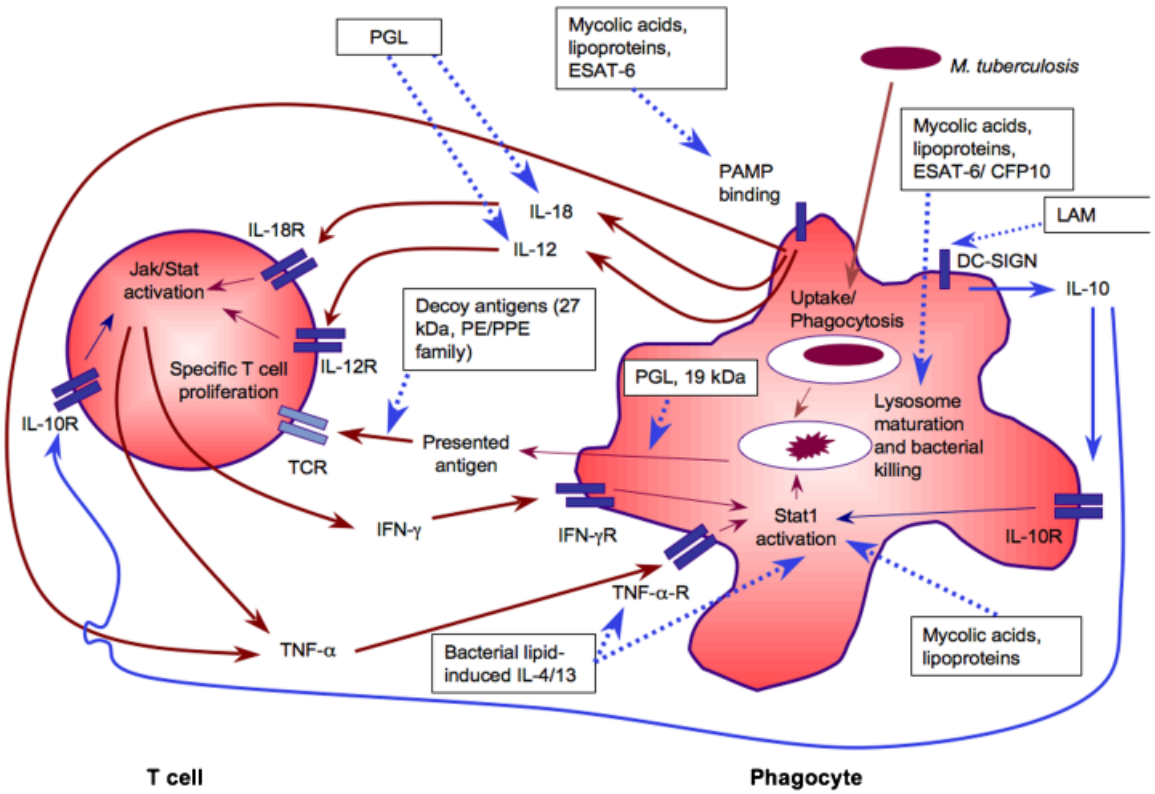

Fig. 2. A simplified schematic, showing the interaction of the infected antigen-presenting cell and an antigen-specific T cell after infection. The key pathways in the host's immune response are shown as solid arrows that can suppress (red) or enhance (blue) bacterial growth, together with the known bacterial products (white boxes, dotted arrows) that can interfere with the host's response.

evolutionary dead end for the bacteria. At the same time, the same immune responses are essential for the host to control bacterial replication. This balance is clearly illustrated by the course of TB in HIV-infected individuals, whose immune deficiency renders them simultaneously more susceptible to fatal bacteremia, and less infectious than normal, because they cavitate less frequently than people with an intact immune response (24).

Thus, because it cannot evade the induction of cell-mediated immunity, *M. tuberculosis* has evolved to survive it, and survive it does – even if the initial infection is successfully controlled, many infected individuals develop a latent infection that can persist for decades (25–28).

INTERACTION WITH MACROPHAGE RECEPTORS

A major component of *M. tuberculosis*'s success as a pathogen rests on its ability to survive within host cells – especially immune cells such as macrophage/monocytes, which are charged with both killing bacteria directly by phagocytosis and priming immune responses by antigen presentation. *M. tuberculosis* does this by interfering with the process of macrophage activation and phagocytosis at virtually every stage (see Fig. 2). This interference starts immediately on contact between the bacteria and the cell's receptors.

Mannose derivatives on the pathogen's surface molecules from pathogenic (but not non-pathogenic) mycobacteria inhibit phagocytosis by activated macrophages (29) and therefore potentially allow the pathogen to target cell types more susceptible to infection. It is known that lipoarabinomannan (LAM) – a major cell wall component of *M. tuberculosis* – can bind to the DC-SIGN molecule, expressed on the surface of dendritic cells. DC-SIGN is crucial to dendritic cell maturation, and LAM binding inhibits this process, decreases IL-12 production and induces dendritic cells to secrete IL-10 (30, 31), which inhibits antigen presentation, expression of

major histocompatibility complex (MHC) molecules and expression of co-stimulatory receptors. Consistent with this, recent studies have found that expression of IL-10 is significantly elevated in TB patients with active disease (32–34).

In addition, the cell wall of *M. tuberculosis* includes many long-chain fatty acids (19, 20, 35, 36) that strongly stimulate host inflammatory responses, leading to granuloma formation (37), upregulation of antigen presentation and subsequent NK and T-cell responses (38, 39). If this immunological process was allowed to develop as described above, the infection would be rapidly eliminated. However, some of those lipoproteins apparently modulate this process to the pathogen's advantage. The 19 kDa lipoprotein of *M. tuberculosis* interacts with host APCs via TLR1/2 (40, 41), but instead of activating protective immunity, this leads to inhibition of cytokine production [reducing the expression of over a third of the interferon (IFN)-γ-activated genes (42)], and reduced antigen-processing and MHC II expression (42–44). This lipoprotein appears to be a virulence factor (45) that reduces overall immunity to the bacterium in mice (46). ESAT-6 has a similar effect, also operating through TLR-2 (47). This – and similar molecules – may contribute to the virulence of epidemic Beijing strains of *M. tuberculosis* in humans by inducing higher levels of IL-4 and IL-13 than non-epidemic strains (48, 49). TLR2/4 ligation was once considered crucial to the inflammatory response to mycobacteria (50, 51), but now it appears more like interference in IFN-γ-signaling via TLR signaling is also a potential virulence mechanism (52). It has even been suggested that by turning the expression of proteins on or off, such as the 19 kDa decoy molecule, *M. tuberculosis* may evade immune surveillance during the latent phase of infection (42, 44, 53), while still allowing the initiation of inflammatory immune responses leading to tissue destruction and cavitation during acute infection or reactivation.

PHAGOCYTOSIS, KILLING AND ANTIGEN PRESENTATION BY MACROPHAGES

Once taken up, the bacteria begin to disrupt the mechanisms of phagosome maturation, creating an intracellular compartment that lacks the acidic, hydrolytic environment needed to kill the bacteria and that resembles in many ways an early endosome. However, fusion with other vesicles and membrane remodeling and trafficking still occurs, allowing *M. tuberculosis* to acquire necessary nutrients and export its own proteins (54–56).

M. tuberculosis interference with phagosomal maturation

A wide range of genes is involved in this process. The functions of some are as yet unknown, but putative transporters, iron-scavenging molecules and lipid-synthesizing molecules are all apparently important (36, 55, 57–59) in preventing normal phagosome maturation. Blocking the accumulation of ATPases and GTPases in the vacuole interferes with the cell's ability to sense the maturation of the phagosome and phagosome function such as for the decrease in pH needed to kill the bacteria (60). The ESAT-6/CFP10 and SecA1/2 proteins on *M. tuberculosis* are virulence factors that interfere with this process (61–63). This process is also dependent at least to some extent on blocking of a calmodulin-dependent Ca^{2+} flux by multiple pathogen-derived molecules (55, 58, 64). Lipids such as trehalose dimycolate can interfere with membrane trafficking, preventing phagosome maturation and surface expression of MHC molecules and co-stimulators; this interference can, to some degree, be prevented by reactive nitrogen intermediates – explaining why activated phagocytes are less susceptible to *M. tuberculosis*-induced inhibitory effects (65–67). Some phagosome-function-inhibiting lipids, such as mannose-capped lipoarabinomannan (ManLAM) (35, 36, 56), appear to be mimics of host phosphatidylinositols, whose presence on the surface of the vacuole normally indicates a maturation state (54, 57). Other molecules such as LRG-47 (54, 68) also interfere with tracking and control of the phagocytic vesicle. Finally, the expression by *M. tuberculosis* of a eukaryotic-like serine/threonine protein kinase G can inhibit phagosome–lysosome fusion. The abundance of known (and presumably unknown) genes involved in altering phagosome maturation and trafficking indicates that interfering with this is a major survival strategy for *M. tuberculosis* (54–57, 64). By holding the

phagosome in a 'non-maturing state,' *M. tuberculosis* prevents fusion with late endsomal/lysosomal vesicles while retaining access to early endosomal vesicles, through which the pathogen can gain access to essential nutrients and cations (especially iron).

M. tuberculosis interference with antigen presentation

In those instances where the phagocyte succeeds in lysing the bacteria, and generating antigens for presentation, the effect may be blunted by the generation of IL-10 and the reduction in cell surface molecules involved in presentation, as noted above. In addition, it has been suggested that *M. tuberculosis* may reduce the efficacy of any immune response induced, by expressing 'decoy' molecules, which stimulate a Th1 immune response that is antigen-specific, but ultimately ineffective. For example, the 27 kDa lipoprotein of *M. tuberculosis* induces a strong IFN-γ secretion, but in animal models at least, these responses are not protective, and, in fact, appear to promote bacterial growth (69, 70). The highly polymorphic PE-PGRS and PPE MPTR gene families have also been suggested to be a source of antigenic variation in *M. tuberculosis*, and TB patients often mount significant immune responses to PGRS proteins (71, 72). Thus, decoy proteins may in part explain why TB patients often have substantial IFN-γ responses to *M. tuberculosis* antigens, and yet are not protected.

ACTIVATION OF THE ADAPTIVE IMMUNE RESPONSE

This modulation of host responses goes beyond intracellular trafficking and has obvious implications for vaccine design. It has been suggested that invasion of phagocytes that are not yet activated is important for the bacteria's survival because exposure of macrophages to IFN-γ and/or tumor necrosis factor (TNF)-α before – but not after – infection decreases the ability of pathogenic mycobacteria to inhibit phagosome maturation and function (54) at least partially by upregulating the production of reactive oxygen and nitrogen derivatives (65, 73–76). However, the production of these cytokines is dependent on activating the adaptive arm of the immune response, which we will discuss in the next sections.

Bridging the gap between innate and adaptive immunity – unconventional T cells

Most individuals respond initially to *M. tuberculosis* infection by producing IFN-γ, and it has been hypothesized that the unconventional T-cell subsets [γδ, NK-T and CD-1 restricted cells (77, 78)], whose receptors are far less variable than that of T cells restricted by conventional MHC I and II molecules, act as a bridge between the innate and the adaptive immune responses by 'kickstarting' cytokine production (79, 80). It is known that γδ T cells and CD1-restricted T cells expand considerably during the early phases of *M. tuberculosis* infection, (79, 80) and by targeting molecules that conventional T cells do not (such as lipids and glycoproteins), they expand the number of cues that the host immune system can respond to (81). Data from genetic knockout models of unconventional T cells have shown only minor effects (77, 78) and it may be that cytotoxicity against infected APC by TCR+γδ T cells, and amplification of APC function via non-cognate cytokine production in the early phases of infection by TCR- γδ T cells is their primary function (82, 83). By secreting IFN-γ, they may help activate APCs – boosting the expression of MHC and costimulatory molecules – and amplifying IL-12 and IL-18 production, resulting in a positive feedback loop for IFN-γ production (82). The importance of IL-12 is highlighted by the observation that gene polymorphisms can affect susceptibility to TB, protection being associated with genotypes leading to high production, and vice versa, while functional mutations in the IL-12 receptor are associated with extreme susceptibility to mycobacterial disease (84, 85). Control of IL-12 expression is key to the expansion and activation of IFN-γ-secreting CD4T cells, which (even more than activation of CD8T cells) is most crucial for immunity to TB, as shown by the susceptibility of animals or patients defective in CD4T cell function or IFN-γ expression or recognition (86–90).

Role of the adaptive immune response in controlling *M. tuberculosis*

While CD4T cells apparently contribute more to the early IFN-γ response, CD8T cells are considered to become more important in the later

phases of disease – possibly via cytotoxic activity and/or IFN-γ production (91–93). Activating Th1 responses has thus been a major objective for the vaccines under development. However, *M. tuberculosis* seems to have developed the ability to subvert the host's immune response, in part by directly countering Th1 function and development. Live bacteria or *M. tuberculosis* cell wall extracts can inhibit some of the downstream effects of IFN-γ, although the mechanism is not yet fully defined (94–96), so that even if IFN-γ is produced, its activity may be reduced. In addition, IFN-γ recall responses are generally reduced in patients with advanced TB (97), while IL-4 is elevated (98–100) and the level of IL-4 gene expression appears to correlate with both the disease severity in TB patients (98, 99) and the risk of subsequent disease in healthy but TB-exposed individuals (101, 102). The observation that the IFN-γ/IL-4 ratio increases in most patients during therapy, but decreases in contacts who become ill, suggests that this state is directly related to the disease (102). Consistent with this is the observation that increased production of splice variants that antagonize IL-4 activity (such as IL-4δ2) appears to be characteristic of individuals who are controlling TB in its latent stage (103) [and the IL-4δ2/IL-4 ratio increases during treatment of TB patients (102), indicating that it is associated with decreased pathology]. Similar observations have also been made in animal models of TB (104). Thus, cell wall components such as phosphoglycolipids or the 19 kDa antigen, which induce IL-4 and IL-13 production, may act as potent virulence factors in clinical strains (36, 48, 49). Likewise, other factors such as LAM binding to the DC-SIGN receptor on the surface of DC may inhibit IFN-γ production and function by inducing IL-10 (30, 31, 34). A poor prognosis in TB is associated with a low IFN-γ/IL-10 ratio just as seen for IFN-γ/IL-4 (102, 105, 106). Altering the balance between IFN-γ and IL-4 or IL-10 production and function thus seems to be a second major survival strategy for *M. tuberculosis*.

An equally important molecule for protection is TNF-α (107), as shown by the rapid reactivation of latent *M. tuberculosis* infection in people treated with TNF-α receptor antagonists (108, 109). The expression of TNF-α is associated with protection in animal models (110, 111), but in the presence of elevated levels of IL-4, TNF-α appears to promote tissue damage rather than protection (112, 113). In addition, infection with *M. tuberculosis*, but not avirulent mycobacteria, promotes the shedding of TNF-α receptors by infected macrophages [(114, 115) and author's unpublished data], which can then serve as soluble antagonists. This paints a picture similar to that seen for IFN-γ: that *M. tuberculosis* can target both gene expression of IFN-γ and TNF-α and also affect their downstream signal induction. Perhaps not surprisingly, in light of the earlier discussions, TNF-α blockade also seems to have a negative effect on phagosome maturation (116). Thus, *M. tuberculosis* seems to have multiple mechanisms targeted toward inhibiting both IFN-γ and TNF-α function and production, and this inhibition has negative consequences for the development of the bactericidal phagosome and the expansion of an effective adaptive immune response. It has another anti-protective function as well, and this is discussed below.

CELL DEATH AND IMMUNOPATHOLOGY

If activation of the cell-mediated immune response is insufficient to eliminate the pathogen, the host has one last option – removal of the infected cells. This can occur by two processes – either apoptosis or necrosis. It has been suggested that apoptosis is a method whereby the host can remove infected cells (117, 118) while minimizing cell death in adjacent, uninfected cells, thus decreasing tissue destruction (119). In support of this are reports showing that resolving granulomas are rich in apoptotic cells and that reduced apoptotic capacity is associated with an inability to control *M. tuberculosis* infection (120). TNF-α is a potent inducer of cell death by apoptosis (121). Necrosis, on the other hand, is associated with the lysis of the infected cell, release of viable *M. tuberculosis* and damage to the surrounding tissue (119). The center of large unresolved granulomas often becomes necrotic, and as mentioned above in the section on immunopathology, this tissue destruction is an essential feature in the spread of *M. tuberculosis*.

It should thus come as no surprise that there is a substantial body of evidence from both

in vitro and *in vivo* studies indicating that virulent *M. tuberculosis* (but not avirulent mycobacteria) can inhibit apoptosis and that this may represent an escape mechanism whereby the pathogen can avoid the death of its host cell by apoptosis (and the internalized bacteria along with it as the apoptotic cell is digested) (122–129). Recent work suggests that *M. tuberculosis* can actively promote necrosis over apoptosis, consistent with the idea that this is a survival/virulence mechanism for the bacteria (130–133). Supporting this hypothesis, studies indicate that elevated levels of necrosis are associated with genetic susceptibility to *M. tuberculosis* in mice (134) or virulence of human-derived clinical isolates (135) and that control of apoptosis via CD43/TNF-α inflammatory responses is important for control of *M. tuberculosis* (136). Some of the genes involved have already been identified. Knock-ins of the nuoG gene conferred on avirulent mycobacteria both the ability to inhibit apoptosis and increased virulence in mice, while its deletion rendered *M. tuberculosis* less able to inhibit apoptosis of infected human monocytes (137). Our own data (Abebe *et al*, unpublished data) suggest that IL-4 plays a role here too, by promoting the expression of multiple anti-apoptotic genes (including Caspase 8 and Fas) and by antagonizing the effect of TNF-α.

Taken in total, these studies indicate that *M. tuberculosis* is able to interfere with almost every stage of the host's immune response and provide some insight into why it is such an effective pathogen. As mentioned above, countering these complex strategies in the design of novel vaccines is a daunting task requiring the activation of the correct response against the correct antigenic targets.

TB VACCINE STRATEGIES – SUBUNIT VACCINES AND RECOMBINANT BCG VACCINES

Selecting antigenic targets for vaccines

For decades, it was believed that only living vaccines (like BCG) could generate the long-lived response necessary to combat *M. tuberculosis* infection and this had a major influence on the search for immunologically relevant TB antigens (138). However, in 1994, Andersen and colleagues, and subsequently other labs, reported the protective effect of vaccination with culture-filtrate proteins (CFPs) prepared from log-phase *M. tuberculosis* cultures in mice and guinea pigs, and demonstrated that the protection was transferable by CD4+ T cells (138). The demonstration that non-living vaccines based on secreted proteins could effectively protect against subsequent *M. tuberculosis* infection in animal models led to the initiation of extensive antigen discovery programs that aimed to identify crucial antigenic molecules. The initial antigens were isolated from filtrates of cultures of actively growing bacteria, which led to the hypothesis that proteins secreted by living bacilli in the phagosome might be the first antigens to be presented to the immune system in the early phase of infection, and consequently an immune response toward these proteins might be more effective at stimulating a protective immune response (138, 139). Antigens from culture filtrates such as ESAT-6, Ag85A/B and TB10.4 have demonstrated good protective efficacy when used as vaccines against an acute infection with *M. tuberculosis*, and these antigens are presently in clinical trials where the aim is to boost BCG-induced immunity (140–143). However, as noted above, the ability of *M. tuberculosis* to develop a latent infection allows it to outlast an immune response generated by vaccination early in life. Moreover, the vaccines in clinical development so far were all derived from actively replicating bacteria, and have all been assessed as prophylactic vaccines (140–143). The primary measure of their efficiency has been their ability to restrict early bacterial growth and dissemination. Preliminary studies suggest that they may have limited activity against dormant bacilli. This is not particularly surprising, as *M. tuberculosis* is able to establish latency and survive in an intracellular habitat for many years by making major changes in gene expression and, therefore, presumably in the antigenic repertoire presented to the immune system. More recent vaccine development strategies are therefore testing the assumption that this change in the antigenic repertoire should be reflected in the vaccines administered to individuals harboring a latent infection. The obvious conclusion is that such vaccines should contain antigens specifically expressed by the dormant bacteria, and this has spurred detailed studies of the gene expression pattern in these bacteria.

How does the dormant *M. tuberculosis* bacteria differ from the actively growing bacteria?

An effective vaccine against *M. tuberculosis* needs to consider the complexity of *M. tuberculosis*' lifestyle. Exposure to *M. tuberculosis* often results in lifelong infection due to the large range of evasion mechanisms deployed by the bacterium. The acute phase of *M. tuberculosis* infection is characterized by rapid bacterial growth and the development of an initial immune response dominated by recognition of secreted bacterial antigens (138, 139, 144, 145). Macrophages and lymphocytes migrate to the site of infection, resulting in the formation of granulomas in the lungs. In the majority of cases, the infection is brought under control by the immune system – even if the pathogen is not eliminated. However, the bacterium responds to the hostile environment of the host and enters a stage (often referred to as dormancy or latency) characterized by a drastically altered metabolism and a significant change in gene expression (146–149). It is unclear at present whether the bacteria in this stage are truly dormant: it is more likely that they persist through limited but continuous replication, or perhaps as a continuum of active and less-active forms (150). The outcome is a latent stage of infection without clinical symptoms that may last for many years or even decades. Latency is a dynamic process in which bacterial outgrowth is controlled by the immune response and, as described above, the bacteria attempt to subvert that immune response. This is a delicate balance that can change at any point (e.g., immunosuppression by HIV), leading to rapid bacterial replication and clinical reactivation of TB (3, 108, 151, 152). Considering the phenotypic change of the bacterium during the different stages of *M. tuberculosis* infection, it is most likely that a successful vaccine against TB may need to induce immune recognition of a broad spectrum of bacterial antigens.

Until recently, little was known about the conditions that induce dormancy and the bacterial response to those conditions. It has been known that control of bacterial replication in animal models requires the production of IFN-γ, TNF-α and nitric oxide (76, 87, 88, 103, 107, 108, 110, 151) and that exposure of the bacteria or bacterially infected cells to these agents *in vitro* or to conditions thought to reflect the conditions inside the granuloma such as limited access to iron, oxygen or nutrients leads to a drastic down-regulation of genes that are highly recognized by TB patients in the early phase of infection (146, 147). Mimicking these conditions and inducing bacterial dormancy *in vitro* has been the subject of intensive research in recent years. O_2 depletion has been the most comprehensively studied and provides a link between the avascular environment of the encapsulated granuloma and the capacity of *M. tuberculosis* to adapt to hypoxic conditions. Wayne and colleagues demonstrated, in a series of important studies, that a gradual depletion of O_2 changes bacterial respiration toward nitrate reduction and induces significant metabolic, chromosomal and structural changes in the bacteria consistent with dormancy (153–155). Recent work using whole genome microarrays has identified >200 genes whose expressions are rapidly altered by defined hypoxic conditions and has identified the dosR regulon that consists of 48 genes (156, 157). The dosR regulon is up-regulated by bacterial sensing of low, non-toxic concentrations of NO and appears to prepare *M. tuberculosis* for dormancy (158). Similarly, other conditions thought to reflect *in vivo* infection, such as growth in activated macrophages or within artificial granulomas, has been demonstrated to up-regulate the dosR genes, and an analogous switch in gene expression during chronic infection of mice has been seen (159). Hypoxia-driven dormancy seems to be reversible, as provision of O_2, even after long periods of hypoxia-induced bacteriostasis, results in resuscitation and bacterial replication. Recent data suggest that synchronous resuscitation of the surviving dormant bacteria may be promoted by pheromone-like substances (the so-called resuscitation-promoting factors) secreted from slowly replicating bacteria and expressed in *M. tuberculosis*-infected patients (160, 161). Some of these substances may also promote bacterial spreading and transmission by dissolving the macrophage cell wall through lysozyme-like activity (162).

Nutrient starvation is another factor expected to be encountered by the bacteria *in vivo* and therefore has been used *in vitro* by Duncan and colleagues to induce a state of non-replicating persistence with decreased respiration. Proteome and microarray analysis demonstrated

that a large number of transcriptional changes occurred, but interestingly, although some of the DosR genes were also up-regulated by starvation, the overall pattern differed significantly from that induced by hypoxia, which would suggest the involvement of a regulon different from DosR (147). Many of these changes appeared to involve lipid metabolism, consistent with earlier findings that long-term survival in the murine lung requires that *M. tuberculosis* express isocitrate lyase, an enzyme essential for the metabolism of fatty acids and for virulence *in vivo* (163). Importantly, this gene was necessary for replication of the bacteria in the late stage of infection in normal mice, whereas bacteria with a disruption of the gene still multiplied in IFN-γ knockout mice. This suggests that the metabolism of *M. tuberculosis in vivo* is profoundly influenced by the host response to infection. It is possible that activated macrophages are more easily able to deprive the bacteria of nutrients [perhaps by resisting changes to phagosome trafficking – (55, 65, 117)] and that the bacteria switch their metabolism to fatty acid degradation in response to this. This hypothesis is supported by the examination of the transcription profile of *M. tuberculosis* grown in activated murine macrophages or in the lungs of infected mice, which indicates that *M. tuberculosis* adapts to immune activation by expressing fatty acid-degrading enzymes and secreting siderophores to facilitate the acquisition of iron (157). This finding underscores the complexity of the bacterial transcriptional response to the multiple environmental signals encountered during its intracellular lifestyle and recent work (discussed in the last section of this chapter) is focusing on how to design vaccines that target the bacteria in its dormant phase.

While the antigens used in vaccines are crucial, it is important to stress that any vaccine against infection with *M. tuberculosis* should induce the correct response against the antigens used. This is particularly important, because, as discussed above, it appears that *M. tuberculosis* has developed the ability to divert immune responses away from those that confer optimal protection and to change its protein expression according to the immune pressure that it is under – including the expression of proteins to directly interfere with the host's immune response and so-called decoy proteins such as the 27 kDa antigen (69, 70).

New targets for vaccine development

Improved understanding of antigen expression patterns has led to a new phase in the intense research on subunit vaccines for TB. Subunit vaccines offer several significant advantages over BCG: first and foremost is the ability to produce a defined product, including antigens expressed by the bacteria in different phases of the infection (discussed in detail below), second is the ability to choose a delivery system that stimulates specifically the kind of immune response – a Th1 dominated response – needed and finally, because they need not be restricted in their growth (or are designed not to require growth in the host) by prior immunity to mycobacteria, their activity in individuals sensitized by environmental mycobacteria or BCG should not be impacted. In a highly cited study, six different atypical mycobacteria strains isolated from soil and sputum samples from Karonga district in Northern Malawi (a region in which BCG vaccination has no effect against pulmonary TB) were investigated in the mouse model. Two of these strains from the *Mycobacterium avium* complex were found to block BCG activity completely. Importantly, the efficacy of a subunit vaccine (in this case, the Ag85B-ESAT-6 fusion discussed below) was completely unaffected by prior sensitization (17). This makes subunit vaccines highly attractive for the boosting strategy. In addition, most subunit vaccines under development use either replication-deficient vectors, or are non-living, meaning that they pose no threat even in HIV-positive individuals. This makes them suitable for vaccination programs in TB-endemic regions, where the TB and HIV epidemics are ever more closely intertwined.

The vaccines being developed fall into two categories. The first is vaccines aimed at replacing BCG, conferring longer and/or more effective protection. At present, it is unlikely that a subunit vaccine can replace BCG in the near future, due to the latter's low cost, safety record and extensive use worldwide, and this 'BCG replacement' vaccine strategy is therefore mostly focused on recombinant BCG or attenuated *M. tuberculosis* vaccines.

The second strategy involves vaccines designed to be administered to already BCG-vaccinated individuals to further boost (and

hopefully prolong) the BCG-induced immunity. Compared with recombinant mycobacterial vaccines, where it is unclear whether such an attenuated vaccine is virulent enough to overcome the existing anti-mycobacterial immunity due to earlier exposure to environmental mycobacteria or a prior BCG vaccination, subunit vaccines do not appear to be affected by – and may even benefit from – existing anti-mycobaterial immunity. Therefore, the obvious choice is to use the mycobacterial vaccines for priming, and subunit vaccines as boosters, allowing designers of boosting vaccines to take advantage of the prevalence of BCG vaccination and the likelihood that this will persist at least for the foreseeable future. However, because a vaccine administered as a booster to adolescents or older children may also be given to individuals who did not receive the BCG vaccine, or who received an ineffective BCG vaccination (incorrectly administered, or with a vaccine that was too old or incorrectly stored), a booster vaccine should also be able to prime an effective immune response. As a result, all of the vaccines currently in clinical trials were initially screened in animal models for the ability to prime a protective immune response at least as efficacious as BCG (141, 143). Because booster vaccines by definition will be administered later in life, the assumption that two billion people are latently infected with *M. tuberculosis* means that any booster vaccine will also of necessity be administered to large numbers of latently infected individuals. This raises the question of safety and any such vaccine will need to be rigorously screened for safety in *M. tuberculosis*-infected individuals. However, it also raises the following question – can we design a vaccine that can help people who are already infected, either because they did not receive a primary vaccination or because it did not prevent a latent infection (not an unlikely scenario in the case of BCG-vaccinated individuals)? Mathematical modeling suggests that a post-exposure vaccine effective at preventing disease in latently infected individuals would cause a significant decrease in the number of new cases in the short term, but that over time, a combination of pre- and post-exposure vaccine would have a larger effect (164). The ideal approach would therefore be a single vaccine that is effective against both acute and latent infection, i.e. a vaccine that can counteract *M. tuberculosis* in different stages of the infection. However, no such 'multistage' vaccine currently exists (165, 166).

CONCLUDING REMARKS

This review has touched on the very complex topic of *M. tuberculosis*–host interaction and focused on the interactions that are most relevant for vaccine design. While it is clearer than ever that designing a vaccine that can cope with the many strategies that *M. tuberculosis* has evolved to escape the host's immune response will be complex, there remain reasons to be optimistic. The first new vaccines against *M. tuberculosis* in half a century are in clinical trials and more candidate vaccines, designed to also protect against reactivation of latent TB, are on their way. New adjuvants, effective at stimulating cell-mediated responses and apparently safe in humans, are also in trials. Phase II trials are already underway with two vaccines and at least two more are expected to reach that stage over the next year. At the same time, more advanced vaccines, which show activity against the latent form of the disease in animal models, are already in late preclinical stages. We are learning more and more about the lifestyle of *M. tuberculosis* – and in this, as so much else, knowledge is power. As we dissect the immune response against *M. tuberculosis*, and the pathogen's response to that response, we are becoming capable of designing vaccine strategies that should allow us to tip the balance in the host's favor.

REFERENCES

1. Anon. Global Tuberculosis Control – Surveillance, Planning, Financing. WHO: World Health Organization, Geneve, Switzerland, 2008.
2. Kochi A. The global tuberculosis situation and the new control strategy of the World Health Organization. 1991. Bull World Health Organ 2001;79:71–5.
3. Girardi E, Raviglione MC, Antonucci G, Godfrey-Faussett P, Ippolito G. Impact of the HIV epidemic on the spread of other diseases: the case of tuberculosis. AIDS 2000;14 (Suppl 3):S47–56.
4. Selwyn PA, Hartel D, Wasserman W, Drucker E. Impact of the AIDS epidemic on morbidity and mortality among intravenous drug users in a

New York City methadone maintenance program. Am J Public Health 1989;79:1358–62.
5. Perneger TV, Sudre P, Lundgren JD, Hirschel B. Does the onset of tuberculosis in AIDS predict shorter survival? Results of a cohort study in 17 European countries over 13 years. AIDS in Europe Study Group. BMJ 1995;311:1468–71.
6. Reid A, Scano F, Getahun H, Williams B, Dye C, Nunn P, et al. Towards universal access to HIV prevention, treatment, care, and support: the role of tuberculosis/HIV collaboration. Lancet Infect Dis 2006;6:483–95.
7. Whalen C, Horsburgh CR, Hom D, Lahart C, Simberkoff M, Ellner J. Accelerated course of human immunodeficiency virus infection after tuberculosis. Am J Respir Crit Care Med 1995; 151:129–35.
8. Donald PR, Schaaf HS. Old and new drugs for the treatment of tuberculosis in children. Paediatr Respir Rev 2007;8:134–41.
9. Lipman M, Breen R. Immune reconstitution inflammatory syndrome in HIV. Curr Opin Infect Dis 2006;19:20–5.
10. Shelburne SA III, Hamill RJ The immune reconstitution inflammatory syndrome. AIDS Rev 2003;5:67–79.
11. Jassal M, Bishai WR. Extensively drug-resistant tuberculosis. Lancet Infect Dis 2009;1:19–30.
12. Colditz GA, Berkey CS, Mosteller F, Brewer TF, Wilson ME, Burdick E, et al. The efficacy of bacillus Calmette–Guerin vaccination of newborns and infants in the prevention of tuberculosis: meta-analyses of the published literature. Pediatrics 1995;96:29–35.
13. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA 1994;271:698–702.
14. Andersen P, Doherty TM. The success and failure of BCG – implications for a novel tuberculosis vaccine. Nat Rev Microbiol 2005;3: 656–62.
15. Leung CC, Tam CM, Chan SL, Chan-Yeung M, Chan CK, Chang KC. Efficacy of the BCG revaccination programme in a cohort given BCG vaccination at birth in Hong Kong. Int J Tuberc Lung Dis 2001;5:717–23.
16. Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, de Brito SC, et al. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. Lancet 2005;366: 1290–5.
17. Brandt L, Feino Cunha J, Weinreich Olsen A, Chilima B, Hirsch P, Appelberg R, et al. Failure of the *Mycobacterium bovis* BCG vaccine: some species of environmental mycobacteria block multiplication of BCG and induction of protective immunity to tuberculosis. Infect Immun 2002;70:672–8.
18. Gonzalez-Juarrero M, Turner OC, Turner J, Marietta P, Brooks JV, Orme IM. Temporal and spatial arrangement of lymphocytes within lung granulomas induced by aerosol infection with *Mycobacterium tuberculosis*. Infect Immun 2001;69:1722–8.
19. Korf J, Stoltz A, Verschoor J, De Baetselier P, Grooten J. The *Mycobacterium tuberculosis* cell wall component mycolic acid elicits pathogen-associated host innate immune responses. Eur J Immunol 2005;35:890–900.
20. Quesniaux V, Fremond C, Jacobs M, Parida S, Nicolle D, Yeremeev V, et al. Toll-like receptor pathways in the immune responses to mycobacteria. Microbes Infect 2004;6:946–59.
21. Stenger S, Modlin RL. Control of *Mycobacterium tuberculosis* through mammalian Toll-like receptors. Curr Opin Immunol 2002;14:452–7.
22. Brosch R, Pym AS, Gordon SV, Cole ST. The evolution of mycobacterial pathogenicity: clues from comparative genomics. Trends Microbiol 2001;9:452–8.
23. de Jonge MI, Brosch R, Brodin P, Demangel C, Cole ST. Tuberculosis: from genome to vaccine. Expert Rev Vaccines 2005;4:541–51.
24. Aaron L, Saadoun D, Calatroni I, Launay O, Memain N, Vincent V, et al. Tuberculosis in HIV-infected patients: a comprehensive review. Clin Microbiol Infect 2004;10:388–98.
25. Andersen P, Doherty TM, Pai M, Weldingh K. The prognosis of latent tuberculosis: can disease be predicted? Trends Mol Med 2007;13:175–82.
26. Manabe YC, Bishai WR. Latent *Mycobacterium tuberculosis*-persistence, patience, and winning by waiting. Nat Med 2000;6:1327–9.
27. Morrison J, Pai M, Hopewell PC. Tuberculosis and latent tuberculosis infection in close contacts of people with pulmonary tuberculosis in low-income and middle-income countries: a systematic review and meta-analysis. Lancet Infect Dis 2008;8:359–68.
28. Stewart GR, Robertson BD, Young DB. Tuberculosis: a problem with persistence. Nat Rev Microbiol 2003;1:97–105.
29. Stokes RW, Norris-Jones R, Brooks DE, Beveridge TJ, Doxsee D, Thorson LM. The glycan-rich outer layer of the cell wall of *Mycobacterium tuberculosis* acts as an antiphagocytic capsule limiting the association of the bacterium with macrophages. Infect Immun 2004;72: 5676–86.
30. Appelmelk BJ, van Die I, van Vliet SJ, Vandenbroucke-Grauls CM, Geijtenbeek TB, van Kooyk Y. Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. J Immunol 2003;170:1635–9.

31. van Kooyk Y, Geijtenbeek TB. DC-SIGN: escape mechanism for pathogens. Nat Rev Immunol 2003;3:697–709.
32. Jang S, Uzelac A, Salgame P. Distinct chemokine and cytokine gene expression pattern of murine dendritic cells and macrophages in response to *Mycobacterium tuberculosis* infection. J Leukoc Biol 2008;84:1264–70.
33. Olobo JO, Geletu M, Demissie A, Eguale T, Hiwot K, Aderaye G, et al. Circulating TNF-alpha, TGF-beta, and IL-10 in tuberculosis patients and healthy contacts. Scand J Immunol 2001;53:85–91.
34. Redpath S, Ghazal P, Gascoigne NR. Hijacking and exploitation of IL-10 by intracellular pathogens. Trends Microbiol 2001;9:86–92.
35. Brennan PJ. Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. Tuberculosis (Edinburgh) 2003;83:91–7.
36. Briken V, Porcelli SA, Besra GS, Kremer L. Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response. Mol Microbiol 2004;53:391–403.
37. Sugawara I, Yamada H, Mizuno S, Li CY, Nakayama T, Taniguchi M. Mycobacterial infection in natural killer T cell knockout mice. Tuberculosis (Edinburgh) 2002;82:97–104.
38. Hunter RL, Olsen MR, Jagannath C, Actor JK. Multiple roles of cord factor in the pathogenesis of primary, secondary, and cavitary tuberculosis, including a revised description of the pathology of secondary disease. Ann Clin Lab Sci 2006;36:371–86.
39. Ryll R, Kumazawa Y, Yano I. Immunological properties of trehalose dimycolate (cord factor) and other mycolic acid-containing glycolipids – a review. Microbiol Immunol 2001;45:801–11.
40. Sugawara I, Yamada H, Li C, Mizuno S, Takeuchi O, Akira S. Mycobacterial infection in TLR2 and TLR6 knockout mice. Microbiol Immunol 2003;47:327–36.
41. Takeda K, Takeuchi O, Akira S. Recognition of lipopeptides by Toll-like receptors. J Endotoxin Res 2002;8:459–63.
42. Pai RK, Pennini ME, Tobian AA, Canaday DH, Boom WH, Harding CV. Prolonged toll-like receptor signaling by *Mycobacterium tuberculosis* and its 19-kilodalton lipoprotein inhibits gamma interferon-induced regulation of selected genes in macrophages. Infect Immun 2004;72:6603–14.
43. Fortune SM, Solache A, Jaeger A, Hill PJ, Belisle JT, Bloom BR, et al. *Mycobacterium tuberculosis* inhibits macrophage responses to IFN-gamma through myeloid differentiation factor 88-dependent and -independent mechanisms. J Immunol 2004;172:6272–80.
44. Noss EH, Pai RK, Sellati TJ, Radolf JD, Belisle J, Golenbock DT, et al. Toll-like receptor 2-dependent inhibition of macrophage class II MHC expression and antigen processing by 19-kDa lipoprotein of *Mycobacterium tuberculosis*. J Immunol 2001;167:910–8.
45. Henao-Tamayo M, Junqueira-Kipnis AP, Ordway D, Gonzales-Juarrero M, Stewart GR, Young DB, et al. A mutant of *Mycobacterium tuberculosis* lacking the 19-kDa lipoprotein Rv3763 is highly attenuated in vivo but retains potent vaccinogenic properties. Vaccine 2007;25:7153–9.
46. Yeremeev VV, Lyadova IV, Nikonenko BV, Apt AS, Abou-Zeid C, Inwald J, et al. The 19-kD antigen and protective immunity in a murine model of tuberculosis. Clin Exp Immunol 2000;120:274–9.
47. Pathak SK, Basu S, Basu KK, Banerjee A, Pathak S, Bhattacharyya A, et al. Direct extracellular interaction between the early secreted antigen ESAT-6 of *Mycobacterium tuberculosis* and TLR2 inhibits TLR signaling in macrophages. Nat Immunol 2007;8:610–8.
48. Manca C, Reed MB, Freeman S, Mathema B, Kreiswirth B, Barry CE III, et al. Differential monocyte activation underlies strain-specific *Mycobacterium tuberculosis* pathogenesis. Infect Immun 2004;72:5511–4.
49. Reed MB, Domenech P, Manca C, Su H, Barczak AK, Kreiswirth BN, et al. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature 2004;431:84–7.
50. Jo EK, Yang CS, Choi CH, Harding CV. Intracellular signalling cascades regulating innate immune responses to Mycobacteria: branching out from Toll-like receptors. Cell Microbiol 2007;9:1087–98.
51. Salgame P. Host innate and Th1 responses and the bacterial factors that control *Mycobacterium tuberculosis* infection. Curr Opin Immunol 2005;17:374–80.
52. Reiling N, Ehlers S, Holscher C. MyDths and un-TOLLed truths: sensor, instructive and effector immunity to tuberculosis. Immunol Lett 2008;116:15–23.
53. Fulton SA, Reba SM, Pai RK, Pennini M, Torres M, Harding CV, et al. Inhibition of major histocompatibility complex II expression and antigen processing in murine alveolar macrophages by *Mycobacterium bovis* BCG and the 19-kilodalton mycobacterial lipoprotein. Infect Immun 2004;72:2101–10.
54. Deretic V, Singh S, Master S, Harris J, Roberts E, Kyei G, et al. *Mycobacterium tuberculosis* inhibition of phagolysosome biogenesis and autophagy as a host defence mechanism. Cell Microbiol 2006;8:719–27.

55. Russell DG. *Mycobacterium tuberculosis*: here today, and here tomorrow. Nat Rev Mol Cell Biol 2001;2:569–77.
56. Steinberg BE, Grinstein S. Pathogen destruction versus intracellular survival: the role of lipids as phagosomal fate determinants. J Clin Invest 2008;118:2002–11.
57. Chua J, Vergne I, Master S, Deretic V. A tale of two lipids: *Mycobacterium tuberculosis* phagosome maturation arrest. Curr Opin Microbiol 2004;7:71–7.
58. Connolly SF, Kusner DJ. The regulation of dendritic cell function by calcium-signaling and its inhibition by microbial pathogens. Immunol Res 2007;39:115–27.
59. Rohde K, Yates RM, Purdy GE, Russell DG. *Mycobacterium tuberculosis* and the environment within the phagosome. Immunol Rev 2007;219:37–54.
60. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, et al. Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase. Science 1994;263:678–81.
61. Hinchey J, Lee S, Jeon BY, Basaraba RJ, Venkataswamy MM, Chen B, et al. Enhanced priming of adaptive immunity by a proapoptotic mutant of *Mycobacterium tuberculosis*. J Clin Invest 2007;117:2279–88.
62. Hou JM, D'Lima NG, Rigel NW, Gibbons HS, McCann JR, Braunstein M, et al. ATPase activity of *Mycobacterium tuberculosis* SecA1 and SecA2 proteins and its importance for SecA2 function in macrophages. J Bacteriol 2008;190:4880–7.
63. Tan T, Lee WL, Alexander DC, Grinstein S, Liu J. The ESAT-6/CFP-10 secretion system of *Mycobacterium* marinum modulates phagosome maturation. Cell Microbiol 2006;8:1417–29.
64. Vergne I, Chua J, Lee HH, Lucas M, Belisle J, Deretic V. Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA 2005;102:4033–8.
65. Axelrod S, Oschkinat H, Enders J, Schlegel B, Brinkmann V, Kaufmann SH, et al. Delay of phagosome maturation by a mycobacterial lipid is reversed by nitric oxide. Cell Microbiol 2008;10:1530–45.
66. Indrigo J, Hunter RL Jr., Actor JK. Cord factor trehalose 6,6′-dimycolate (TDM) mediates trafficking events during mycobacterial infection of murine macrophages. Microbiology 2003;149:2049–59.
67. Kan-Sutton C, Jagannath C, Hunter RL Jr. Trehalose 6,6′-dimycolate on the surface of *Mycobacterium tuberculosis* modulates surface marker expression for antigen presentation and costimulation in murine macrophages. Microbes Infect 2009;1:40–8.
68. MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. Science 2003;302:654–9.
69. Hovav AH, Bercovier H. Pseudo-rationale design of efficient TB vaccines: lesson from the mycobacterial 27-kDa lipoprotein. Tuberculosis (Edinburgh) 2006;86:225–35.
70. Hovav AH, Mullerad J, Davidovitch L, Fishman Y, Bigi F, Cataldi A, et al. The *Mycobacterium tuberculosis* recombinant 27-kilodalton lipoprotein induces a strong Th1-type immune response deleterious to protection. Infect Immun 2003;71:3146–54.
71. Singh PP, Parra M, Cadieux N, Brennan MJ. A comparative study of host response to three *Mycobacterium tuberculosis* PE_PGRS proteins. Microbiology 2008;154:3469–79.
72. Zheng H, Lu L, Wang B, Pu S, Zhang X, Zhu G, et al. Genetic basis of virulence attenuation revealed by comparative genomic analysis of *Mycobacterium tuberculosis* strain H37Ra versus H37Rv. PLoS ONE 2008;3:e2375.
73. Davis AS, Vergne I, Master SS, Kyei GB, Chua J, Deretic V. Mechanism of inducible nitric oxide synthase exclusion from mycobacterial phagosomes. PLoS Pathog 2007;3:e186.
74. Nathan C. Role of iNOS in human host defense. Science 2006;312:1874–5; author reply -5.
75. Schon T, Elmberger G, Negesse Y, Pando RH, Sundqvist T, Britton S. Local production of nitric oxide in patients with tuberculosis. Int J Tuberc Lung Dis 2004;8:1134–7.
76. Green SJ, Scheller LF, Marletta MA, Seguin MC, Klotz FW, Slayter M, et al. Nitric oxide: cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. Immunol Lett 1994;43:87–94.
77. Ladel CH, Blum C, Dreher A, Reifenberg K, Kaufmann SH. Protective role of gamma/delta T cells and alpha/beta T cells in tuberculosis. Eur J Immunol 1995;25:2877–81.
78. Ladel CH, Hess J, Daugelat S, Mombaerts P, Tonegawa S, Kaufmann SH. Contribution of alpha/beta and gamma/delta T lymphocytes to immunity against *Mycobacterium bovis* bacillus Calmette Guerin: studies with T cell receptor-deficient mutant mice. Eur J Immunol 1995;25:838–46.
79. Schaible UE, Kaufmann SH. CD1 molecules and CD1-dependent T cells in bacterial infections: a link from innate to acquired immunity? Semin Immunol 2000;12:527–35.
80. Ulrichs T, Porcelli SA. CD1 proteins: targets of T cell recognition in innate and adaptive immunity. Rev Immunogenet 2000;2:416–32.
81. Behar SM, Porcelli SA. CD1-restricted T cells in host defense to infectious diseases. Curr Top Microbiol Immunol 2007;314:215–50.

82. Caccamo N, Sireci G, Meraviglia S, Dieli F, Ivanyi J, Salerno A. Gammadelta T cells condition dendritic cells in vivo for priming pulmonary CD8T cell responses against *Mycobacterium tuberculosis*. Eur J Immunol 2006;36:2681–90.
83. Dieli F, Caccamo N, Meraviglia S, Ivanyi J, Sireci G, Bonanno CT, et al. Reciprocal stimulation of gammadelta T cells and dendritic cells during the anti-mycobacterial immune response. Eur J Immunol 2004;34:3227–35.
84. de Jong R, Altare F, Haagen IA, Elferink DG, Boer T, van Breda Vriesman PJ, et al. Severe mycobacterial and Salmonella infections in interleukin-12 receptor-deficient patients. Science 1998;280:1435–8.
85. Tso HW, Lau YL, Tam CM, Wong HS, Chiang AK. Associations between IL12B polymorphisms and tuberculosis in the Hong Kong Chinese population. J Infect Dis 2004;190:913–9.
86. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene-disrupted mice. J Exp Med 1993;178:2243–7.
87. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. J Exp Med 1993;178:2249–54.
88. Ottenhoff TH, Verreck FA, Hoeve MA, van de Vosse E. Control of human host immunity to mycobacteria. Tuberculosis (Edinburgh) 2005;85:53–64.
89. Saunders BM, Frank AA, Orme IM, Cooper AM. CD4 is required for the development of a protective granulomatous response to pulmonary tuberculosis. Cell Immunol 2002;216:65–72.
90. Scanga CA, Mohan VP, Yu K, Joseph H, Tanaka K, Chan J, et al. Depletion of CD4(+) T cells causes reactivation of murine persistent tuberculosis despite continued expression of interferon gamma and nitric oxide synthase 2. J Exp Med 2000;192:347–58.
91. Lazarevic V, Nolt D, Flynn JL. Long-term control of *Mycobacterium tuberculosis* infection is mediated by dynamic immune responses. J Immunol 2005;175:1107–17.
92. Sud D, Bigbee C, Flynn JL, Kirschner DE. Contribution of CD8+T cells to control of *Mycobacterium tuberculosis* infection. J Immunol 2006;176:4296–314.
93. Woodworth JS, Behar SM. *Mycobacterium tuberculosis*-specific CD8+T cells and their role in immunity. Crit Rev Immunol 2006;26:317–52.
94. Kincaid EZ, Ernst JD. *Mycobacterium tuberculosis* exerts gene-selective inhibition of transcriptional responses to IFN-gamma without inhibiting STAT1 function. J Immunol 2003;171:2042–9.
95. Ting LM, Kim AC, Cattamanchi A, Ernst JD. *Mycobacterium tuberculosis* inhibits IFN-gamma transcriptional responses without inhibiting activation of STAT1. J Immunol 1999;163:3898–906.
96. Manca C, Tsenova L, Freeman S, Barczak AK, Tovey M, Murray PJ, et al. Hypervirulent *M. tuberculosis* W/Beijing strains upregulate type I IFNs and increase expression of negative regulators of the Jak-Stat pathway. J Interferon Cytokine Res 2005;25:694–701.
97. Sodhi A, Gong J, Silva C, Qian D, Barnes PF. Clinical correlates of interferon gamma production in patients with tuberculosis. Clin Infect Dis 1997;25:617–20.
98. Jalapathy KV, Prabha C, Das SD. Correlates of protective immune response in tuberculous pleuritis. FEMS Immunol Med Microbiol 2004;40:139–45.
99. Jimenez-Martinez MC, Linares M, Baez R, Montano LF, Martinez-Cairo S, Gorocica P, et al. Intracellular expression of interleukin-4 and interferon-gamma by a *Mycobacterium tuberculosis* antigen-stimulated CD4+CD57+T-cell subpopulation with memory phenotype in tuberculosis patients. Immunology 2004;111:100–6.
100. Roberts T, Beyers N, Aguirre A, Walzl G. Immunosuppression during active tuberculosis is characterized by decreased interferon- gamma production and CD25 expression with elevated forkhead box P3, transforming growth factor-beta, and interleukin-4 mRNA levels. J Infect Dis 2007;195:870–8.
101. Ordway DJ, Costa L, Martins M, Silveira H, Amaral L, Arroz MJ, et al. Increased Interleukin-4 production by CD8 and gammadelta T cells in health-care workers is associated with the subsequent development of active tuberculosis. J Infect Dis 2004;190:756–66.
102. Wassie L, Demissie A, Aseffa A, Abebe M, Yamuah L, Tilahun H, et al. Ex vivo cytokine mRNA levels correlate with changing clinical status of ethiopian TB patients and their contacts over time. PLoS ONE 2008;3:e1522.
103. Demissie A, Abebe M, Aseffa A, Rook G, Fletcher H, Zumla A, et al. Healthy individuals that control a latent infection with *Mycobacterium tuberculosis* express high levels of Th1 cytokines and the IL-4 antagonist IL-4delta2. J Immunol 2004;172:6938–43.
104. Rhodes SG, Sawyer J, Whelan AO, Dean GS, Coad M, Ewer KJ, et al. Is interleukin-4delta3 splice variant expression in bovine tuberculosis a marker of protective immunity? Infect Immun 2007;75:3006–13.
105. Hussain R, Talat N, Shahid F, Dawood G. Longitudinal tracking of cytokines after acute exposure to tuberculosis: association of distinct

cytokine patterns with protection and disease development. Clin Vaccine Immunol 2007;14: 1578–86.
106. Sahiratmadja E, Alisjahbana B, de Boer T, Adnan I, Maya A, Danusantoso H, et al. Dynamic changes in pro- and anti-inflammatory cytokine profiles and gamma interferon receptor signaling integrity correlate with tuberculosis disease activity and response to curative treatment. Infect Immun 2007;75:820–9.
107. Jacobs M, Togbe D, Fremond C, Samarina A, Allie N, Botha T, et al. Tumor necrosis factor is critical to control tuberculosis infection. Microbes Infect 2007;9:623–8.
108. Anon. Tuberculosis associated with blocking agents against tumor necrosis factor-alpha – California, 2002–2003. Morb Mortal Wkly Rep 2004;53:683–6.
109. Gomez-Reino JJ, Carmona L, Valverde VR, Mola EM, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. Arthritis Rheum 2003;48:2122–7.
110. Flynn JL, Goldstein MM, Chan J, Triebold KJ, Pfeffer K, Lowenstein CJ, et al. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. Immunity 1995;2:561–72.
111. Ogawa T, Uchida H, Kusumoto Y, Mori Y, Yamamura Y, Hamada S. Increase in tumor necrosis factor alpha- and interleukin-6-secreting cells in peripheral blood mononuclear cells from subjects infected with *Mycobacterium tuberculosis*. Infect Immun 1991;59:3021–5.
112. Seah GT, Rook GA. Il-4 influences apoptosis of mycobacterium-reactive lymphocytes in the presence of TNF-alpha. J Immunol 2001;167: 1230–7.
113. Sharma S, Bose M. Role of cytokines in immune response to pulmonary tuberculosis. Asian Pac J Allergy Immunol 2001;19:213–9.
114. Lawn SD, Rudolph D, Wiktor S, Coulibaly D, Ackah A, Lal RB. Tuberculosis (TB) and HIV infection are independently associated with elevated serum concentrations of tumour necrosis factor receptor type 1 and beta2-microglobulin, respectively. Clin Exp Immunol 2000;122:79–84.
115. Tsao TC, Hong J, Li LF, Hsieh MJ, Liao SK, Chang KS. Imbalances between tumor necrosis factor-alpha and its soluble receptor forms, and interleukin-1beta and interleukin-1 receptor antagonist in BAL fluid of cavitary pulmonary tuberculosis. Chest 2000;117:103–9.
116. Harris J, Hope JC, Keane J. Tumor necrosis factor blockers influence macrophage responses to *Mycobacterium tuberculosis*. J Infect Dis 2008;198:1842–50.
117. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. Cell 2004;119:753–66.
118. Kremer L, Estaquier J, Brandt E, Ameisen JC, Locht C. *Mycobacterium bovis* Bacillus Calmette Guerin infection prevents apoptosis of resting human monocytes. Eur J Immunol 1997;27: 2450–6.
119. Bocchino M, Galati D, Sanduzzi A, Colizzi V, Brunetti E, Mancino G. Role of mycobacteria-induced monocyte/macrophage apoptosis in the pathogenesis of human tuberculosis. Int J Tuberc Lung Dis 2005;9:375–83.
120. Patel NR, Zhu J, Tachado SD, Zhang J, Wan Z, Saukkonen J, et al. HIV impairs TNF-alpha mediated macrophage apoptotic response to *Mycobacterium tuberculosis*. J Immunol 2007; 179:6973–80.
121. Stenger S. Immunological control of tuberculosis: role of tumour necrosis factor and more. Ann Rheum Dis 2005;64 (Suppl 4):24–8.
122. Keane J, Balcewicz-Sablinska MK, Remold HG, Chupp GL, Meek BB, Fenton MJ, et al. Infection by *Mycobacterium tuberculosis* promotes human alveolar macrophage apoptosis. Infect Immun 1997;65:298–304.
123. Kremer L, Estaquier J, Wolowczuk I, Biet F, Ameisen JC, Locht C. Ineffective cellular immune response associated with T-cell apoptosis in susceptible *Mycobacterium bovis* BCG-infected mice. Infect Immun 2000;68:4264–73.
124. Loeuillet C, Martinon F, Perez C, Munoz M, Thome M, Meylan PR. *Mycobacterium tuberculosis* subverts innate immunity to evade specific effectors. J Immunol 2006;177:6245–55.
125. Ozeki Y, Kaneda K, Fujiwara N, Morimoto M, Oka S, Yano I. In vivo induction of apoptosis in the thymus by administration of mycobacterial cord factor (trehalose 6,6′-dimycolate). Infect Immun 1997;65:1793–9.
126. Placido R, Mancino G, Amendola A, Mariani F, Vendetti S, Piacentini M, et al. Apoptosis of human monocytes/macrophages in *Mycobacterium tuberculosis* infection. J Pathol 1997;181:31–8.
127. Rios-Barrera VA, Campos-Pena V, Aguilar-Leon D, Lascurain LR, Meraz-Rios MA, Moreno J, et al. Macrophage and T lymphocyte apoptosis during experimental pulmonary tuberculosis: their relationship to mycobacterial virulence. Eur J Immunol 2006;36:345–53.
128. Roger PM, Bermudez LE. Infection of mice with *Mycobacterium avium* primes CD8+lymphocytes for apoptosis upon exposure to macrophages. Clin Immunol 2001;99:378–86.
129. Watson VE, Hill LL, Owen-Schaub LB, Davis DW, McConkey DJ, Jagannath C, et al. Apoptosis in *Mycobacterium tuberculosis* infection in

mice exhibiting varied immunopathology. J Pathol 2000;190:211–20.
130. Budak F, Uzaslan EK, Cangur S, Goral G, Oral HB. Increased pleural soluble Fas ligand (sFasL) levels in tuberculosis pleurisy and its relation with T-helper type 1 cytokines. Lung 2008;186:337–43.
131. Gan H, Lee J, Ren F, Chen M, Kornfeld H, Remold HG. *Mycobacterium tuberculosis* blocks crosslinking of annexin-1 and apoptotic envelope formation on infected macrophages to maintain virulence. Nat Immunol 2008;9:1189–97.
132. Mustafa T, Mogga SJ, Mfinanga SG, Morkve O, Sviland L. Significance of Fas and Fas ligand in tuberculous lymphadenitis. Immunology 2005;114:255–62.
133. Porcelli SA, Jacobs WR Jr. Tuberculosis: unsealing the apoptotic envelope. Nat Immunol 2008;9:1101–2.
134. Kramnik I. Genetic dissection of host resistance to *Mycobacterium tuberculosis*: the sst1 locus and the Ipr1 gene. Curr Top Microbiol Immunol 2008;321:123–48.
135. Park JS, Tamayo MH, Gonzalez-Juarrero M, Orme IM, Ordway DJ. Virulent clinical isolates of *Mycobacterium tuberculosis* grow rapidly and induce cellular necrosis but minimal apoptosis in murine macrophages. J Leukoc Biol 2006;79:80–6.
136. Randhawa AK, Ziltener HJ, Stokes RW. CD43 controls the intracellular growth of *Mycobacterium tuberculosis* through the induction of TNF-alpha-mediated apoptosis. Cell Microbiol 2008;10:2105–17.
137. Velmurugan K, Chen B, Miller JL, Azogue S, Gurses S, Hsu T, et al. *Mycobacterium tuberculosis* nuoG is a virulence gene that inhibits apoptosis of infected host cells. PLoS Pathog 2007;3:e110.
138. Andersen P. Effective vaccination of mice against *Mycobacterium tuberculosis* infection with a soluble mixture of secreted mycobacterial proteins. Infect Immun 1994;62:2536–44.
139. Andersen P. The T cell response to secreted antigens of *Mycobacterium tuberculosis*. Immunobiology 1994;191:537–47.
140. Doherty TM, Dietrich J, Billeskov R. Tuberculosis subunit vaccines: from basic science to clinical testing. Expert Opin Biol Ther 2007;7:1539–49.
141. Doherty TM, Olsen AW, Weischenfeldt J, Huygen K, D'Souza S, Kondratieva TK, et al. Comparative analysis of different vaccine constructs expressing defined antigens from *Mycobacterium tuberculosis*. J Infect Dis 2004;190:2146–53.
142. Ly LH, McMurray DN. Tuberculosis: vaccines in the pipeline. Expert Rev Vaccines 2008;7:635–50.
143. Williams A, Hatch GJ, Clark SO, Gooch KE, Hatch KA, Hall GA, et al. Evaluation of vaccines in the EU TB Vaccine Cluster using a guinea pig aerosol infection model of tuberculosis. Tuberculosis (Edinburgh) 2005;85:29–38.
144. Abou-Zeid C, Smith I, Grange JM, Ratliff TL, Steele J, Rook GA. The secreted antigens of *Mycobacterium tuberculosis* and their relationship to those recognized by the available antibodies. J Gen Microbiol 1988;134:531–8.
145. Andersen P, Askgaard D, Gottschau A, Bennedsen J, Nagai S, Heron I. Identification of immunodominant antigens during infection with *Mycobacterium tuberculosis*. Scand J Immunol 1992;36:823–31.
146. Bacon J, Marsh PD. Transcriptional responses of *Mycobacterium tuberculosis* exposed to adverse conditions in vitro. Curr Mol Med 2007;7:277–86.
147. Betts JC, Lukey PT, Robb LC, McAdam RA, Duncan K. Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. Mol Microbiol 2002;43:717–31.
148. Hampshire T, Soneji S, Bacon J, James BW, Hinds J, Laing K, et al. Stationary phase gene expression of *Mycobacterium tuberculosis* following a progressive nutrient depletion: a model for persistent organisms? Tuberculosis (Edinburgh) 2004;84:228–38.
149. Sherman DR, Voskuil M, Schnappinger D, Liao R, Harrell MI, Schoolnik GK. Regulation of the *Mycobacterium tuberculosis* hypoxic response gene encoding alpha-crystallin. Proc Natl Acad Sci USA 2001;98:7534–9.
150. Garton NJ, Waddell SJ, Sherratt AL, Lee SM, Smith RJ, Senner C, et al. Cytological and transcript analyses reveal fat and lazy persister-like bacilli in tuberculous sputum. PLoS Med 2008;5:e75.
151. Chan J, Flynn J. The immunological aspects of latency in tuberculosis. Clin Immunol 2004;110:2–12.
152. Scanga CA, Mohan VP, Joseph H, Yu K, Chan J, Flynn JL. Reactivation of latent tuberculosis: variations on the Cornell murine model. Infect Immun 1999;67:4531–8.
153. Starck J, Kallenius G, Marklund BI, Andersson DI, Akerlund T. Comparative proteome analysis of *Mycobacterium tuberculosis* grown under aerobic and anaerobic conditions. Microbiology 2004;150:3821–9.
154. Wayne LG, Hayes LG. An in vitro model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. Infect Immun 1996;64:2062–9.
155. Woolhiser L, Tamayo MH, Wang B, Gruppo V, Belisle JT, Lenaerts AJ, et al. In vivo adaptation

of the Wayne model of latent tuberculosis. Infect Immun 2007;75:2621–5.
156. Rustad TR, Harrell MI, Liao R, Sherman DR. The enduring hypoxic response of *Mycobacterium tuberculosis*. PLoS ONE 2008;3:e1502.
157. Waddell SJ, Butcher PD. Microarray analysis of whole genome expression of intracellular *Mycobacterium tuberculosis*. Curr Mol Med 2007;7:287–96.
158. Voskuil MI, Visconti KC, Schoolnik GK. *Mycobacterium tuberculosis* gene expression during adaptation to stationary phase and low-oxygen dormancy. Tuberculosis (Edinburgh) 2004;84:218–27.
159. Talaat AM, Ward SK, Wu CW, Rondon E, Tavano C, Bannantine JP, et al. Mycobacterial bacilli are metabolically active during chronic tuberculosis in murine lungs: insights from genome-wide transcriptional profiling. J Bacteriol 2007;189:4265–74.
160. Davies AP, Dhillon AP, Young M, Henderson B, McHugh TD, Gillespie SH. Resuscitation-promoting factors are expressed in *Mycobacterium tuberculosis*-infected human tissue. Tuberculosis (Edinburgh) 2008;88:462–8.
161. Tufariello JM, Mi K, Xu J, Manabe YC, Kesavan AK, Drumm J, et al. Deletion of the *Mycobacterium tuberculosis* resuscitation-promoting factor Rv1009 gene results in delayed reactivation from chronic tuberculosis. Infect Immun 2006;74:2985–95.
162. Kana BD, Gordhan BG, Downing KJ, Sung N, Vostroktunova G, Machowski EE, et al. The resuscitation-promoting factors of *Mycobacterium tuberculosis* are required for virulence and resuscitation from dormancy but are collectively dispensable for growth in vitro. Mol Microbiol 2008;67:672–84.
163. McKinney JD, Honer zu Bentrup K, Munoz-Elias EJ, Miczak A, Chen B, Chan WT, et al. Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. Nature 2000;406:735–8.
164. Dye C, Williams BG. Eliminating human tuberculosis in the twenty-first century. J R Soc Interface 2008;5:653–62.
165. Doherty TM, Andersen P. Vaccines for tuberculosis: novel concepts and recent progress. Clin Microbiol Rev 2005;18:687–702.
166. Skeiky YA, Sadoff JC. Advances in tuberculosis vaccine strategies. Nat Rev Microbiol 2006;4:469–76.