Immunological protection against tuberculosis

Willem A Hanekom, Brian Abel, Thomas J Scriba

Ninety per cent of humans never develop tuberculosis (TB) disease following infection with *Mycobacterium tuberculosis*. This implies that most of us have inherent immune capacity to protect us against TB. Persons who do develop disease are likely to be overtly immune compromised by HIV infection, or relatively immune compromised by the low socio-economic conditions that characterise many TB patients' backgrounds. From an epidemiological perspective, it is therefore likely that immune suppression, rather than an inherently inadequate immune system, is the most important determinant of predisposition to disease. However, on an individual basis, immunocompetent persons may still develop TB disease, albeit less frequently, and virulence characteristics of the infecting pathogen may confound the scenario.

The immunological basis of development of disease in 10% of people following infection with *M. tuberculosis* remains incompletely understood. This review focuses on what we know, as a brief introduction aimed at non-immunologists. Excellent and more comprehensive reviews on this topic already exist.[1-4]

Innate and adaptive immunity

Our immune systems have two distinct, although interactive, arms. 'Innate' immunity refers to host responses that occur upon first encounter with a pathogen. This type of immunity protects against a broad range of microbial challenges in a nonspecific manner. The many cell types of the innate system include macrophages, which are on the front line of defence against TB (see below). In contrast, 'adaptive' immunity is specific for a pathogen, and is also called 'specific' immunity. After the innate immune system identifies an invading pathogen and attempts to control its early spread, adaptive immunity develops to deal with that specific challenge. This primary adaptive response is designed to control the pathogen, but also results in so-called 'immunological memory'. This means that the encounter is remembered and enables a more rapid and vigorous response when re-infection with the same pathogen occurs. The T lymphocyte is an example of an adaptive immune cell that is central in protection against TB (see below).

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Early innate immune events in the lung

When a person with active pulmonary TB coughs, *M. tuberculosis* is spread into the immediate environment. The pathogen 'hangs' in the air for prolonged periods, within microdroplets. These droplets may be inhaled into peripheral small airways where macrophages, resident in alveolar spaces, ingest the bacilli. Macrophages have special pattern-recognition receptors termed toll-like receptors (TLR), which sense that the phagocytosed material is foreign to the body. Furthermore, the TLRs immediately recognise the nature of the pathogen, and instruct an appropriate immune response. This allows the macrophage to set into motion immune machinery that leads to 'inflammation', a critical early step in controlling the pathogen. Inflammation is mediated by soluble immune messenger molecules, called cytokines, which are secreted by the macrophage to recruit multiple cell types of the innate immune system to the site. These include natural killer cells and γδ T cells, which, in turn, produce cytokines that activate the macrophage to become mycobactericidal.

Epidemiological evidence supports the hypothesis that early killing of *M. tuberculosis* may be successful, as up to 70% of household contacts of TB patients never develop a positive tuberculin skin test, the hallmark of specific immunity. In the other 30%, the innate inflammatory response is probably inadequate for control of the pathogen. In this scenario, *M. tuberculosis* replicates within the macrophage and ultimately causes cell death. Dead macrophages may be taken up by other macrophages, or by another innate immune cell, the dendritic cell,[3] so called because of long processes that resemble dendrites of neurons. Dendritic cells are the most efficient innate immune cells to induce a primary adaptive response. Dendritic cells may either phagocytose dead innate immune cells containing tuberculosis bacilli, or directly ingest *M. tuberculosis* (as illustrated in Fig. 1). TLR triggering of dendritic cells, together with the action of local cytokines, leads to a series of functional and morphological changes of the cell, termed maturation. This activation process is critical for dendritic cells to migrate from the lung parenchyma to regional lymph nodes to initiate specific immunity (see below).

Dendritic cell maturation also enables optimal 'antigen processing' and 'presentation' of the foreign material. Antigen processing refers to a series of events in which engulfed mycobacterial proteins are broken down into short peptides that associate with specialised host-cell proteins, the major histocompatibility complex (MHC) molecules. MHC-peptide complexes are transported from within the cell to its surface, where they may interact with T cells in a process termed antigen presentation (see below). Multiple, complex

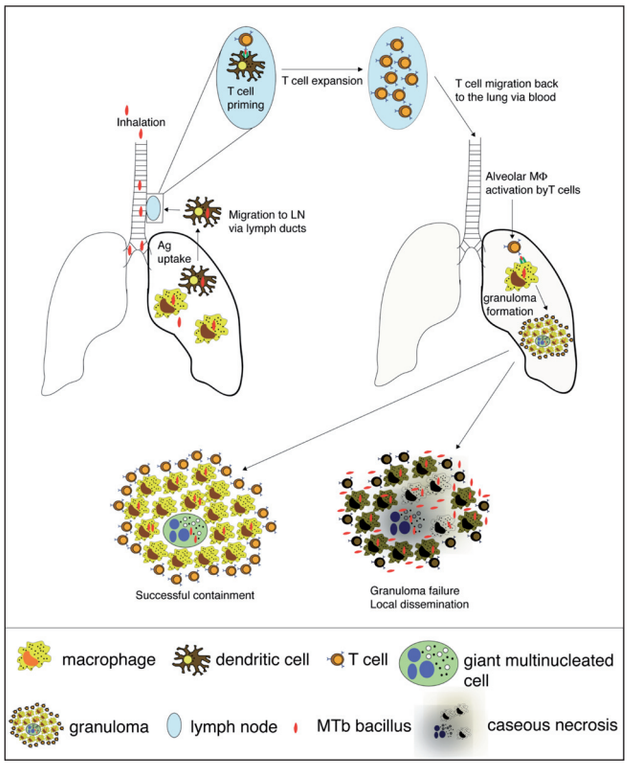

Fig. 1. Events that follow infection with M. tuberculosis. Following inhalation of the bacillus, alveolar macrophages ingest the pathogen. Dendritic cells ingest M. tuberculosis either directly or via ingested dying, infected macrophages. Activated dendritic cells migrate to the draining lymph nodes where naïve mycobacteria-specific T cells are primed to differentiate into antigen-experienced cells. These T cells expand and migrate to the site of infection, the lung, via the blood. In the lung, T cells further activate macrophages and induce the formation of granulomas. Successful granuloma formation and maintenance result in containment of the pathogen. Defective granuloma formation allows mycobacterial growth, and leads to cell death (caseous necrosis), permitting dissemination of bacilli.

mechanisms of antigen processing exist while the pathogen itself has evolved numerous means to subvert these processes (reviewed by Flynn and Chan[2] and Kaufmann and Schaible[3]).

It has been hypothesised that bacilli escaping incompetent macrophages may find their way into blood vessels and result in an early bacteraemia. This bacteraemia happens prior to the development of adaptive immune responses and allows the pathogen to establish niches of infection at remote sites, such as the upper lung, kidneys, spine or meninges. Because a relatively small number of mycobacteria are involved, disease may not result at these sites. Nevertheless, establishing infection niches via this route may be a critical early step for manifestations of extrapulmonary tuberculosis. This bacteraemia contrasts with the blood-borne dissemination that occurs rarely following established lung disease (see below).

Specific immunity develops in regional lymph nodes

Once mature dendritic cells arrive in lymph nodes via lymph ducts, they position themselves in areas of the node where they can encounter an enormous number of T cells, routinely circulating through the node from the blood. A T cell that has never encountered antigen is called naïve. Naïve T cells are preprogrammed to each recognise a specific foreign peptide only, such as those derived from pathogenic organisms. The TB-derived MHC-peptide complexes on dendritic cells are recognised by naïve TB-specific T cells through their T-cell receptor. This results in the formation of an immunological synapse, a stable 'conjugation' which involves interactions between other surface molecules on the cells. Successful synapse formation facilitates optimum T-cell receptor triggering, which, with the action of cytokines such as interleukin-12 (IL-12) secreted by the dendritic cell, results in 'activation' of the T cell. The T cell now starts dividing actively, allowing infrequent TB-specific naïve T cells to expand exponentially. The expanded antigen-experienced T cells will now traffic back to the lung to perform critical functions for control of the infection.

Back in the lung, T cells act and granulomas form

T cells migrate back to the lung via blood. The inflammatory milieu at the initial infection site has also modified blood vessels in the vicinity that now guide T cells to leave the circulation and enter the lung parenchyma. Here, activated T cells recognise TB-derived peptides presented on the surface of infected macrophages and form stable immunological synapses (Fig. 2). Specific T cells activate macrophages more efficiently than innate immune cells early after infection (above), and this activated state may enable enhanced killing of ingested mycobacteria.

Two main groups of T cells exist, namely CD4+ and CD8+ T cells. The majority of T cells at the disease site are CD4+ T cells, also called 'helper' T cells, as their primary role is to produce cytokines that assist and orchestrate other immune cells in the environment. TB-specific CD4+ T cells produce primarily type 1 cytokines, or Th1 cytokines, which include interferon-gamma (IFN-γ), IL-2 and tumour necrosis factor (TNF). IFN-γ is the most critical cytokine for optimal activation of macrophages. CD8+ T cells are also able to secrete these cytokines but, in addition, have the ability to directly kill mycobacteria-containing macrophages. CD8+ T cells accomplish killing through multiple mechanisms, including the production of molecules like perforin and granzymes that literally punch holes in the macrophage membrane. CD8+ T

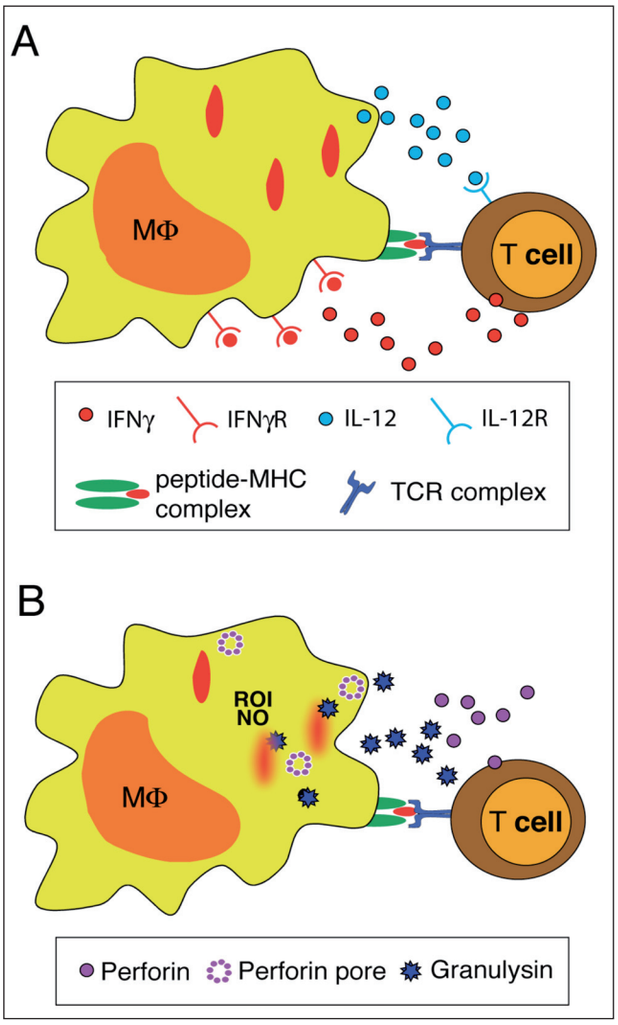

Fig. 2. Presentation of mycobacteria-derived peptides to specific T cells leads to activation of these cells, and ultimately killing of M. tuberculosis by the macrophages. (A) T-cell recognition of peptides presented by the MHC molecules on infected macrophages (MΦ) occurs through the T-cell receptor. Macrophages also secrete the cytokine IL-12, which allows further activation of the T cell, which may ultimately result in release of IFN-γ. This cytokine is important for activating the macrophage. (B) M. tuberculosis within the macrophage is killed through multiple mechanisms. Activation of the macrophage triggers the production of factors such as reactive oxygen intermediates (ROI) and nitric oxide (NO), which may directly kill the bacillus. Alternatively, the infected macrophage may be killed, thus preventing further replication of M. tuberculosis. This is mediated by cytotoxic CD8+ T cells, via the secretion of factors such as perforin, which punch holes into the macrophage membrane. These CD8+ T cells are also able to kill the bacilli within the macrophage by secreting the bactericidal protein granulysin.

cells may also produce the molecule granulysin, which can enter the macrophage via pores to be directly cytotoxic to the ingested mycobacteria.

The interaction between T cells and macrophages is bidirectional. For example, macrophages in the lung produce IL-12 which assists T cells in their function, whereas T-cell-derived IFN-γ activates the macrophages.

In an attempt to contain the infection, multiple T cells and macrophages organise themselves into a classic structure, termed a granuloma (illustrated in Fig. 1). Granulomas comprise macrophages in the centre, surrounded by T cells. Macrophages in granulomas may fuse to form multinucleated giant cells, Langhans cells. Local secretion of the cytokine TNF is required for optimal granuloma structure. The granuloma may result in killing of the organism, but also enable the control of the organisms by merely preventing further replication and forcing the pathogen to enter a state of latency (see below).

Granuloma formation also occurs within regional lymph nodes, to control the organisms that have been carried to this site by infected macrophages.

Unsuccessful containment of the pathogen within granulomas

In about 5% of cases containment within the granuloma is not successful. The bacteria out-compete the immune response, and their intracellular replication causes widespread macrophage death. The term caseous necrosis describes the remains of multiple dead macrophages at the centre of the granuloma. This breakdown may ultimately involve the entire granuloma, allowing mycobacteria to spread to neighbouring tissues. Local spread of the organism, triggering formation of more granulomas, is typical of lung TB. Very extensive granuloma breakdown causing widespread tissue destruction characterises adult TB disease, which is visible on chest radiographs as cavities. In contrast, children very rarely present with lung cavities. The complementary observation that children have paucibacillary disease may support a hypothesis that immune control of TB in this age group is optimal. One reason for this may be that excessive inflammation occurs in adult TB, resulting in immune-mediated tissue destruction. Excessive inflammation may trigger release of inappropriately high levels of cytokines such as TNF. This phenomenon highlights the central paradigm in immune control of TB: a balanced immune response is required, e.g. TNF is required for optimal granuloma formation, but excessive production of the cytokine may be detrimental.

Immune control in children may not necessarily be 'better', as small infants may manifest with severe, disseminated forms of TB disease, including miliary disease and TB meningitis. The immunopathogenesis of miliary disease is relatively simple: local breakdown of a granuloma into a blood vessel causes spread of multiple bacilli to distant organs. The immunopathogenesis of TB meningitis is less clear, but may involve unsuccessful control of bacilli that have lodged on

meninges after the early bacteraemia mentioned above. Alternatively, it may involve later dissemination from the lung or lymph nodes.

Latent disease and reactivation

Successful containment within granulomas results in latent disease, which occurs in up to 95% of cases following infection. As with any manifestation of TB, latency is the result of a dynamic interaction between host and pathogen. The host is able to contain the pathogen, which, in turn, switches to an alternate metabolic state allowing long-term survival. Ability to culture *M. tuberculosis* from 'normal' lung tissue obtained at autopsy of persons who have died from other causes is strong support of long-term survival.[5] The organism is not completely inactive. Rather, it does not replicate and expresses a different set of genes, and the protein products may result in host immune responses to a different set of antigens, compared with active disease or with early time periods after infection.

Reactivation may occur much later in life in about 5% of cases and is the result of disturbance of the host-pathogen equilibrium. The importance of host factors is evidenced by a vastly increased rate of reactivation in HIV-infected people whose CD4+ T cells, critical for protection against disease, are depleted. However, relative immune deficiency, as described above, may also predispose to reactivation. Bacterial factors may also be important, e.g. an undefined trigger may result in the latent pathogen to initiate expression of so-called resuscitation-promoting factors, which allow a switch to the active, non-latent state resulting in replication and ultimately disease.

Immunopathogenesis in HIV-infected persons

Approximately 80% of South Africans with TB are also HIV infected. TB is most common in advanced HIV disease; however, very early in disease progression there is already an increased risk of lung disease. Interestingly, while adult TB disease in early HIV infection resembles that of HIV-uninfected persons, disease during AIDS appears more similar to childhood disease with absence of lung cavitation and a risk of dissemination.

It is thought that the primary immunopathogenic event predisposing to increased risk of TB in HIV infection is CD4+ T-cell depletion. This, in turn, results in multiple secondary immunological defects, which may increase susceptibility. The TB granuloma in more advanced HIV disease is disorganised and unable to contain the bacillus, resulting in local and often distant dissemination.

Antiretroviral therapy may result in partial reconstitution of mycobacteria-specific immunity and will reduce the risk of active disease following infection, but not to the same extent as in HIV-uninfected persons. In latently infected persons, TB disease sometimes manifests only after antiretroviral therapy is commenced. Additionally, active disease may be unmasked or made worse by initiating treatment. These phenomena are called the immune reconstitution inflammatory syndrome (IRIS), and are characterised by the release of large amounts of pro-inflammatory cytokines. The comprehensive immunopathogenesis of TB-associated IRIS remains poorly understood.

TB is a detrimental opportunistic infection for the HIV-infected patient because chronic immune activation driven by TB-induced inflammation may enhance HIV replication. This results in even more immune damage, which, in turn, may prevent control of *M. tuberculosis*.

Critical determinants of immune protection against TB

Lessons learnt from human congenital immune deficiencies may guide our understanding of immune components that are absolutely critical for protection against TB. A group of disorders termed Mendelian Susceptibility to Mycobacterial Disease (MSMD) are associated with severe disease caused by relatively avirulent mycobacteria, including *M. bovis* BCG. These disorders all affect the type 1 cytokine pathway, i.e. the IFN-γ-IL-12 pathway. A range of mutations in the surface receptors for these three cytokines, or in a subunit of IL-12 or in an intracellular signalling molecule of this pathway, have been described.[6,7] We may therefore deduct that the type 1 cytokine pathway, and particularly these three cytokines, are critical for protection against mycobacteria.

The central role of IFN-γ in protection against mycobacteria has led to the development of several commercial assays in which this cytokine is quantified as a readout for mycobacterial immunity, such as the Quantiferon™ test. It is important to note that although currently IFN-γ is probably the best marker of mycobacterial immunity, and is indispensable for protection, mouse experiments have demonstrated that the magnitude of the IFN-γ response may not correlate with vaccination-induced protection.[8,9] The immune correlates of human vaccination-induced or post-infectious protection against TB disease are not known, but future studies will hopefully shed light on this important area.

Several other genetic associations, mostly single nucleotide polymorphisms, with TB disease have been described.[7] In the majority of cases the underlying immune mechanisms predisposing to increased disease risk are not known. It is likely that a combination of genetic and environmental factors determines immunological control of *M. tuberculosis.*

Conclusion

This review is a simplified synopsis of TB immunity. We are aware that a complex interaction of multiple cell types including different innate cells and T-cell subsets other than the conventional ones discussed above, such as regulatory T cells and Th17 cells are important for optimal immunity against mycobacteria. Faced with the escalation of TB incidence worldwide and the emergence of extensively drug-resistant TB (XDR TB), it is critical to gain an understanding of the mechanisms of protection and to delineate immune correlates of this protection. Only then will we be able to optimally design and test new immunotherapeutic interventions such as novel vaccines. An effective vaccine against lung TB may ultimately prove to be the most effective and sustainable intervention in the global TB pandemic.

WAH is supported by the Aeras Global Tuberculosis Vaccine Foundation, the NIH (RO1-AI065653 and NO1-AI-70022), the European and Developing Countries Trials Partnership, the Dana Foundation and the Bill and Melinda Gates Foundation through Grand Challenges in Global Health Grants #37772 and #37885. BA is supported by the National Research Foundation. TJS is a Wellcome Trust Research Training Fellow.

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