Introduction
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As a scourge of humankind throughout recorded history and a disease of poets and romantics, tuberculosis (TB) continues to have a devastating impact on the world’s population. Robert Koch identified *Mycobacterium tuberculosis* (*M. tb*) to be the causative agent of this disease in the year 1882. The development of a vaccine against tuberculosis by Albert Calmette and Camille Gue’rin in 1921, an attenuated *Mycobacterium bovis* strain, bacille Calmette-Gue’rin (BCG), and the discovery of the first antibiotic against tuberculosis, streptomycin, by Selman Waksman in 1943, soon led to the opinion that appropriate control measures had become available for tuberculosis. With the assumption that tuberculosis was controlled in “high-income” countries by vaccination, chemotherapy, hygienic measures, and improved living standards, research on tuberculosis abated.

The emergence of AIDS, increasing incidences of multidrug-resistant (MDR) *M. tb* strains paralleled by increasing migration between the continents and the breakup of the Soviet Union, has brought tuberculosis back to the public health agenda. The immunologic weapon got somewhat blunted, because BCG induces different protective responses across different geographical distributions against pulmonary tuberculosis in adults. Today, tuberculosis is recognized as one of the major microbial killers responsible for 8–9 million new cases annually, of which 1.5–2.0 million die (World Health Organization 2008). Surveillance studies based on positive delayed-type hypersensitivity (DTH) responses to purified protein derivative (PPD) indicate that one third of the world population is infected with *M. tb*, underlining that our immune system is quite efficacious in containing the pathogen but inefficacious in eradicating it. Fortunately, infected individuals have only a 10% lifetime risk of developing disease. However, a vast number of the world population is at risk for reactivated tuberculosis once the immune system
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weakens. This is reflected by the dangerous liaison observed between the human immunodeficiency virus (HIV) and \textit{M. tb}. Co-infection with both pathogens dramatically increases the risk of developing tuberculosis. Add to this, is the rapid emergence and spread of MDR and XDR strains, coupled with increasing the cost for treatment by two orders of magnitude and/or no treatment options at all. Consequently, tuberculosis has drawn the attention of major funding organizations and over the years has resulted in a tremendous boost to our understanding of the biology and immune regulation during infection in animal and human models (Kaufmann 2006).

Over thousands of years microbes and mammals have co-evolved, resulting in extraordinarily sophisticated molecular mechanisms permitting the organisms to survive together. \textit{Mycobacterium tuberculosis} is one of the best examples of successful co-evolution, since the bacilli have infected one third of the human population, but in 90\% of the cases without causing overt disease. This reflects a remarkably clever characteristic of this pathogen – the tubercle bacillus promotes its own survival by allowing the host to endure (Flynn and Chan 2005). Another vital piece of \textit{M. tb} pathogenic proficiency is its use of latent and hence, asymptomatic infection to garner a large toll of world’s population as relatively healthy carriers of the microbe.

\textit{M. tb} is an acid-fast bacillus that is transmitted primarily \textit{via} respiratory route. Infection occurs in the lungs, but the organism can seed any organ \textit{via} haematogenous spread. There are various possible outcomes for a person encountering \textit{M. tb} bacilli. \textbf{Firstly}, although rare, the bacillus might be immediately destroyed by the host’s innate responses. \textbf{Secondly}, a proportion of persons, estimated to be 5 - 10\%, infected with \textit{M. tb} develop acute, active tuberculosis within a finite time frame (1 to 3 years) (Styblo 1980) probably due to lack of initiation of an appropriate immune response. This group presumably lacks the ability to both control the initial infection and develop a protective response in time to prevent disease. \textbf{Finally}, majority of persons infected with \textit{M. tb} have a clinically latent infection; that is, they are able to contain but not eliminate the primary infection. The factors that enable most people to control the initial infection are only partially understood, and it is not clear how \textit{M. tb} persist in the face of a strong immune response.
suffering from the disease expel droplets containing minute number of bacilli. *M. tb* containing aerosols are inhaled into the pulmonary alveoli of the healthy individuals coming in contact of patients. Here, bacteria bind to phagocytic receptors and enter resident alveolar macrophages, dendritic cells and monocytes recruited from the bloodstream to the site of infection. Besides expressing phagocytic receptors, macrophages and dendritic cells also express Toll-like receptors (TLRs) that recognize conserved molecular patterns expressed on pathogens (Medzhitov and Janeway 2000; Kaisho and Akira 2000; Takeda et al., 2003). As depicted in figure 2.2, a small percentage of individuals, despite exposure to *M. tb*, remain uninfected, most likely due to the expression of high innate immunity. However, in the majority of individuals who are exposed to *M. tb*, the innate immune response is not sufficient to contain infection, and Th1 cytokines of the adaptive immune response are necessary to restrict bacterial growth and to mediate protection. The adaptive immunity generated in these people, although protective, nonetheless does not induce sterilizing immunity.

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**Figure 2.2 The natural history of Mycobacterium tuberculosis infection.**

The innate and adaptive immune systems allow most healthy people (> 90%) to control the growth of *M. tuberculosis*, although they harbour latent infection (it is not known whether host immune responses can eliminate infection). Some individuals, especially those with impaired T cell function, develop active tuberculosis (TB), either as primary progression or as a reactivation. (Source: Harding and Boom 2010)
These individuals, therefore, remain latently infected, and are vulnerable to disease reactivation when their immune surveillance weakens or when their immune response is compromised. Reactivation tuberculosis contributes significantly to the morbidity and mortality associated with the disease (Stead 1965; 1967), and is believed to account for a substantial portion of TB cases in HIV-infected individuals (Selwyn et al., 1989). In another small proportion of individuals, infection leads directly to primary tuberculosis due to the failure of their adaptive immune response to control the initial bacterial replication.

2.6.1 Events in the pathogenesis of TB

There are different stages that define the progression of a disease after inhalation of bacilli (Reinout et al., 2002).

First stage: It begins with the inhalation of tubercle bacilli. Alveolar macrophages ingest the bacilli and often destroy them. The destruction of mycobacteria depends on the intrinsic microbicidal capacity of host phagocytes and virulence factors of the ingested mycobacteria.

Second stage (Symbiotic stage): Mycobacteria, which escape the initial intracellular destruction, multiply in the macrophages. This leads to the disruption of macrophages. Blood monocytes and other inflammatory cells are recruited to the lung. These monocytes differentiate into macrophages that again readily ingest but do not destroy the mycobacteria. Bacteria grow logarithmically, and blood-derived macrophages accumulate, but little tissue damage occurs.

Third stage: T-cell immunity develops with antigen-specific T lymphocytes that arrive and proliferate within the early lesions or tubercles and, then, activate macrophages to kill the intracellular mycobacteria. The early logarithmic bacillary growth stops. Central solid necrosis in these primary lesions inhibits extracellular growth of mycobacteria. As a result, infection may become stationary or dormant.

Fourth stage (Post-primary tuberculosis): Disease may progress, and hematogenous dissemination may take place after primary infection, as well as months or years afterwards, under the conditions of failing immune surveillance. Liquefied caseous foci
provide excellent conditions for extracellular growth of *M. tb*. Cavity formation may lead to rupture of nearby bronchi, allowing the bacilli to spread through the airways to other parts of the lung and the outside environment.

### 2.6.2 Granuloma

The progression of a disease is determined at the level of infection site itself. Once the bacterium has gained entry into a macrophage and triggered it to invade the tissue of the lung, the host responds by remodeling the site of infection into a cellular mass, the ‘tubercle’ or granuloma that has given the disease its name. Ghon first defined the tuberculous granuloma in 1912. Granuloma is the hallmark of mycobacterium infections and is the epicenter for the host immune response and bacterial persistence. Its formation serves the host by containing bacteria thereby creating a localized immune response that prevents dissemination. However, the granuloma may also serve the bacteria by ensuring its survival and subsequent disease transmission. Thus, understanding the granuloma microenvironment during latency may not only reveal how *M. tb* survive inside the host for long periods of time, but might also explain why vaccine and therapeutic efforts often fail to result in bacterial clearance.

Infection with *M. tb* follows a relatively well-defined sequence of events. The infectious bacilli are inhaled as droplets from the atmosphere. Exhaled droplets or nuclei are known to remain in the atmosphere for several hours. In lungs, the bacteria are phagocytosed by alveolar macrophages that induce a localized proinflammatory response leading to the recruitment of mononuclear cells from neighbouring blood vessels (figure 2.3). These cells are the building blocks for the granuloma, or tubercle, which is the signature of tuberculosis. A granuloma is an organized collection of immune cells, specifically including a large proportion of macrophages, which occurs in a tissue as a result of chronic unresolved inflammation. The inflammatory stimulus may or may not be infectious, and a number of conditions apart from TB also exhibit granulomas including cryptococcosis, sarcoidosis, leprosy and chronic granulomatous disease.
The TB granuloma consists of a kernel of infected macrophages surrounded by foamy macrophages and other mononuclear phagocytes, with a mantle of lymphocytes in association with a fibrous cuff of collagen and other extracellular matrix components that delineates the periphery of the structure (figure 2.3).

This tissue response typifies the ‘containment’ phase of the infection in which there are no overt signs of disease and the host does not transmit the infection to others. In the later stages, granuloma develops a marked fibrous sheath and number of blood vessels penetrating the structure diminishes markedly. Following a change in the immune status of host (due to old age, malnutrition or co-infection with HIV), the granuloma caseates (decays into a structureless mass of cellular debris), ruptures and spills thousands of viable, infectious bacilli into the airways. This results in the development of a productive cough that facilitates aerosol spread of infectious bacilli.

**Figure 2.3: The pathology of granuloma**
(Source: Russell 2007)

### 2.6.3 Development of TB granuloma

The human tuberculosis granuloma is the product of a robust cellular immune response to bacterial components. It is seen that alveolar macrophages in the airways, following internalization of inhaled bacteria, are stimulated to invade the lung epithelium (Ulrichs and Kaufmann 2006; Flynn and Chan 2005; Algood et al., 2005).
Production of tumour necrosis factor (TNF)-α and inflammatory chemokines from the infected macrophages drives the recruitment of successive waves of neutrophils, natural killer (NK) T cells, CD4$^+$ T cells and CD8$^+$ T cells, each of which produce their own complement of chemokines and cytokines that amplify cellular recruitment and remodeling of the infection site (Algood et al., 2003). This inflammatory cascade is regulated and superceded by a specific cellular immune response that is linked to the production of interferon (IFN)-γ. At this stage, formation of the ‘stable’ granuloma that is responsible for immune containment during the latent, or subclinical, period of the infection becomes recognizable and the stratification of the structure emerges (Ulrichs et al., 2005; Kaplan et al., 2003). However, some studies in macaque monkey indicates a more heterogeneous picture in which caseation is observed even in early lesions and there is marked heterogeneity between the granulomas present in a single host (Lin et al., 2006). More mature-phase granulomas show marked neo-vascularization and develop an extensive fibrotic capsule that delineates margin between the macrophages, granulocytes, foamy macrophages and giant cells, and the lymphocytic infiltrate (Ulrichs and Kaufmann 2006; Kaplan et al., 2003; Dheda et al., 2005).

In late stages, centre of the granuloma loses its vascular appearance and becomes necrotic. In a progressive lesion, the necrosis precedes and facilitates caseation, the granuloma wall breaks down and bacteria are released into the airways, resulting in transmission.

Interestingly, it seems that while the interior of the granuloma harbours few antigen presenting cells (APCs) that contain mycobacterial antigens, the areas immediately surrounding the granuloma exhibit abundant, organized aggregates of APCs and proliferating lymphocytes and are therefore the likely site of active immunity (Ulrichs et al., 2006). The granuloma also allows the chronic maintenance of M. tb in infected macrophages. Granuloma has long been considered to be necessary for the containment of infection but a recent study by Davis and Ramakrishnan suggested that granulomas might promote infection, rather than simply containing it (Davis et al., 2009). The most important cellular player that may be involved in initiating the immune response to TB is
In approximately 5 to 10% of latently infected persons, the infection has the potential to reactivate and cause active tuberculosis (Selwyn et al., 1989).

In response to infection with M. tb, most individuals mount a robust immune response, culminating in the formation of a granulomatous lesion that apparently contains the infection. The host response prevents active disease from occurring, and the bacterium avoids elimination. In most cases, the host response is sufficient to forestall active disease for a lifetime. However, occasionally the immune response fails in some way or the other and the infection reactivates to cause active disease. A constant battle between the host and the mycobacterium is thus being waged, and the outcome depends on many factors, which need to be explored in detail for the complete understanding of disease pathogenesis.

Decades of work have focused on the interaction of this pathogen with its established cellular host, the macrophage, but the identification and regulation of exact process remains to be fully worked out. While the macrophage is clearly important, many evidences suggest that understanding the role of dendritic cells, which are key regulators of immunity, is also a crucial step in identifying new means of controlling this disease. It is imperative to dissect the complex dynamic relationships between host cells and mycobacteria to highlight the new areas of intervention that have not been previously explored. A more complete understanding of the roles played by each component of immune system in protection or exacerbation of tuberculosis, as well as of the bacterium’s weapons to evade those components, will enhance the development of preventive and therapeutic strategies against this enormously successful pathogen.

Between infection and the appearance of first symptoms of the disease, bacteria interact with different microenvironments within the host. The outcome of such host–pathogen interactions are in large part due to selective gene expression at different phases of infection (Krinos et al., 2001). Consequently, understanding bacterial gene expression in vivo is central to our understanding of how bacteria colonize, invade, and interact with or disrupt the normal host cell functions and eventually produce disease. A clear
understanding of the molecular events responsible for establishing and maintaining
tuberculosis will likely lead to improved drug and vaccine design.

With respect to latent tuberculosis, an important area of research is to identify the factors
and mechanisms by which *M. tb* evades host antimicrobial defenses and survive in the
face of a strong immune response. Towards this aspect, many different mechanisms have
already been explored. For example, pathogenic mycobacterial species survive inside
macrophages by arresting the normal maturation of their phagosome, thereby restricting
its acidification to pH 6.4 and limiting fusion with pre-formed lysosomes (Deretic *et al.*, 
2006; Russell 2001; Russell *et al.*, 2005). Various bacterial effector molecules have been
found to play a role in arresting phagosome maturation. These include a lipid phosphatase
(SapM), a tyrosine phosphatase (PtpA) (Bach *et al.*, 2008), a serine/threonine kinase
(PknG) (Walburger *et al.*, 2004), a lipoamide dehydrogenase (LpdC) (Deghmane *et al.*, 
2007) etc. Survival of *M. tb* is not only facilitated by the manipulation of its primary host
cell, but also due to resistance against stresses that the bacteria encounter in
immunologically activated macrophages. Different genes and mechanisms help *M. tb* to
specifically withstand the acidic, nitro-oxidative stresses of the host and help it to
establish its own niche. Thus, a wide range of molecules and mechanism exists that help
the bacteria to survive inside the hostile environment presented by the host.

On these lines, one key aspect of *M. tb* virulence is the secretion of around 600 protein
antigens in the axenic cultures. Over the years, many of these antigens have found use in
diagnostics and as potential ‘vaccine’ candidates. But, despite a wealth of information
available on these antigens, their exact roles at the site of infection and the physiological
relevance of secretion are yet to be ascertained. Many *M. tb* protein and non-protein
antigens have been demonstrated to play roles in immune evasion. In particular, the 19-
kDa antigen has been well characterized to compromise many antibacterial functions of
macrophages in a TLR2-dependent manner (Pennini *et al.*, 2006). Culture filtrate protein
10 kDa (CFP-10), a secretory protein of *M. tb*, is a target of cellular immune response in
mice and humans infected with *M. tb* and serve as important immunodiagnostic markers
for latent TB infection. The interaction studies of CFP-10 with the cells of immune
system especially dendritic cells (DCs) have been found to induce suppressor kind of immune response in context of *M. tb* infection (Balkhi *et al.*, 2004). These studies shed light on the fact that secretion of some of the antigens such as CFP-10 or 19-kDa antigen could be a strategy employed by *M. tb* towards downregulation of protective and pro-inflammatory responses at sites of infection.

In light of the above reports, identification of many more *M. tb* antigens like CFP-10 and the 19-kDa antigen that would be expressed inside macrophages during the course of infection would help in the better understanding of host-pathogen interactions that essentially determine the immune response mounted against the disease. Based on the available findings, the present work focused on a comprehensive study involving the characterization of different antigens that might be involved in regulating the immune responses at various stages of infection.