CHAPTER II

Review of literature

To prove that tuberculosis is caused by the invasion of bacilli and that it is a parasitic disease primarily caused by the growth and multiplication of bacilli, it is necessary to isolate the bacilli from the body, to grow them in pure culture until they are freed from every disease product of the animal organism, and, by introducing isolated bacilli into animals, to reproduce the same morbid condition that is known to follow from inoculation with spontaneously developed tuberculous material.

...Robert Koch
**Chapter II**

**REVIEW OF LITERATURE**

*Tuberculosis as a destroyer of mankind has no equal*

Perhaps no bacterial infection has tormented the human race with as much tenacity and destructive impact as has the ancient disease-Tuberculosis (TB). While TB never disappeared from the developing world, it re-emerged globally from the oblivion and has resurged with a vengeance after the advent of global pandemic of human HIV infection in the 1980’s. Additionally, in the new millennium, the drug resistant forms of TB continue to haunt us (Espinal *et al*., 2001; Corbett *et al*., 2003).

Among adults, TB is the most common cause of death due to any single infectious agent. *Mycobacterium tuberculosis* (*M*. *tuberculosis*), the etiological agent of TB, is one of the oldest and world’s most successful human pathogens (Medlar, 1926; Koch, 1932; Dobson, 1951; Kaplan *et al*., 1974; Sutherland, 1976; Murray, 1990; Dye *et al*., 1999). One third of the world’s population is asymptptomatically infected with *M.tuberculosis* accounting for a staggering two billion people being latently infected. According to World Health Organization (WHO), *M.tuberculosis* is responsible for 8.7 million new TB cases and approximately 1.4 million deaths each year worldwide (WHO, 2012). The TB pandemic is facing additional complications due to the emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) *M.tuberculosis* strains. The deadly synergy between *M.tuberculosis* and HIV has made the situation even more precarious. The impact of this “cursed duet” on human suffering has been enormous. Realizing the gloomy scenario, WHO had declared TB as a “Global Health Emergency” in 1993.

*Mycobacterium tuberculosis* *A pathogen that refuses to be tamed*

*M.tuberculosis* belongs to the family *Mycobacteriaceae* and the order *Actinomycetales*. The generic name *Mycobacterium* was coined by Lehmann and Neumann in 1896 because of its mold like pellicular growth in liquid medium. *M.tuberculosis* colonies on solid media displayed raised, irregular, dry, wrinkled colonies, which are buff colored (Figure 1A). Microscopically, tubercle bacilli are slender, straight or slightly curved rod shaped organisms measuring 2-4 μm in length and 0.2-0.8 μm in breadth occurring singly, in pairs or in small groups (Figure 1B).
The bacilli resist decolorization by 25% sulphuric acid and absolute alcohol for 10 min and hence, these are called acid and alcohol fast bacilli. Acid fastness is based on the integrity of the cell wall. The mycobacterial cell wall is highly complex in nature. It has a high lipid content which accounts for about 60% of the cell wall weight. The cell wall has several distinct layers. The inner layer, overlying the cell membrane is composed of peptidoglycan [murein]. External to the murein is a layer of arabinogalactan, which is covalently linked to a group of long chain fatty acids termed “mycolic acid”. The generation time of \textit{M. tuberculosis} \textit{in vitro} is about 18 hours. Hence, the growth rate is much slower than that of most bacteria.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Colony morphology and acid fast property of \textit{M. tuberculosis}.}
\end{figure}

\textit{M. tuberculosis} has developed a ‘hit-and-stay’ strategy based on the slow growth, long incubation time and delayed onset of disease.

\textbf{Immunopathology of tuberculosis: Life and death in granuloma}

\textit{M. tuberculosis} was originally thought to be an obligate aerobe, but there are numerous experimental indications that the bacterium can grow in microaerophilic environments e.g., in lung granulomas (McCune \textit{et al.}, 1966b; Gillespie \textit{et al.}, 1986; Realini \textit{et al.}, 1998; Glickman and Jacobs, 2001). \textit{M. tuberculosis} is transmitted by aerosols originating from the lungs of persons suffering from active TB (Ottenhoff and Kaufmann, 2012). \textit{M. tuberculosis}-loaded aerosols are inhaled by individuals nearby and reach the alveoli of their lungs, where they are taken up by various cell types, including alveolar macrophages, interstitial macrophages, local dendritic cells (DCs) and epithelial cells (Figure 2).
There are 3 possible outcomes. (i) Immediate eradication of *M. tuberculosis* by the pulmonary immune system. (ii) Infection transforms into TB. This is hallmarked by the formation of central caseous necrotic lesions, filled with extracellular infectious *M. tuberculosis* and cell debris (Hopewell, 1994; McMurray *et al.*, 1996; Turner *et al.*, 2003; Reece and Kaufmann, 2012). *M. tuberculosis* is able to persist inside otherwise hostile host cells, notably professional phagocytes, by employing different immune evasion strategies (Hassett and Cohen, 1989). These strategies include the inhibition of phagosome maturation, inhibition of autophagy, inhibition of apoptosis, egression into the cytosol, blocking of MHC antigen processing and presentation and the inhibition of interferon (IFN)γ-receptor signaling, all of which help *M. tuberculosis* to evade host defense and establish disease (Deretic, 2006; Rohde *et al.*, 2007; Sahiratmadja *et al.*, 2007). In the diseased individual, *M. tuberculosis* is no longer contained because caseation of the lesion results in dissemination and transmission of
M. tuberculosis (Figure 2). (iii) Infection does not transform into disease because M. tuberculosis is contained inside granulomas. After inhalation, M. tuberculosis is engulfed by alveolar macrophages and DCs. In draining lymph nodes, these cells present mycobacterial antigens to different T cell populations. Antigen presentation probably involves cross-priming, allowing transfer of mycobacterial antigens from infected macrophages to dendritic cells. Antigen-specific CD4+ T cells, CD8+ T cells and CD1-restricted T cells participate in protection. Most importantly, macrophages are activated by IFN-γ and TNF-α. In addition, T cells may kill mycobacteria present in macrophages by means of perforin and granulysin. This allows M. tuberculosis to establish dormant, non-replicating or persisting organisms that remain in a metabolic low activity state in infected cells and represent a potential reservoir of bacteria that can resuscitate later on in life (Figure 2).

Evolution of granuloma: a double edged sword

Following initial deposition of M. tuberculosis in the lung, macrophages and other immune cells like dendritic cells, epithelial cells are recruited at the site of infection during the early innate response to infection (Braude, 1951; Ramakrishnan, 2012). The resulting cellular infiltrates become organized as primary granulomas. These are highly dynamic structures from which cells can rapidly influx and efflux. At a later stage, when adaptive immunity has been initiated, specific CD4+ and CD8+ T-lymphocytes start infiltrating the granuloma; this leads to the formation of larger, well-organized, solid granulomas in which M. tuberculosis cells are contained mostly centrally (Figure 3). During maturation into productive granulomas, mononuclear phagocytes differentiate into macrophages and become highly activated, aggregating into multinucleated giant cells and larger epithelioid-like cells that contain tightly interdigitated cell membranes. This barricade-like structure walls off the organism and limits further dissemination to additional sites of infection (Cardona et al., 2000; Reece and Kaufmann, 2012). If control of infection is not balanced optimally, the central regions of the granulomas become necrotic and later caseous thus allowing outgrowth of high numbers of M. tuberculosis organisms (Turner et al., 2003; Russell et al., 2009; Dorhoi et al., 2011). The pathogen then disseminates from lungs to the pulmonary lymph nodes via hematogenous spread and then appears in spleens within ~3 weeks post infection (McMurray, 1994, 2001). This is followed by reseeding of the lung by M. tuberculosis by ~4 weeks to form secondary granulomas. When the liquefying granulomas also damage airway linings, infected material is discharged into the airways. At this stage, TB infection has become contagious. Thus, immunity to M. tuberculosis is a two-edged sword: it protects the human host against
Review of literature

disseminating infection, but also facilitates transmission of TB to contacts. Therefore, TB vaccines not only need to induce optimal immunity to *M. tuberculosis*, but also a balanced response that favors protective and avoids pathogenic mechanisms (Smith, 1994; Ulrichs and Kaufmann, 2006).

**Figure 3: Different tubercle phenotypes during active human tuberculosis.**
(a) Early-stage tubercles of grey and half-translucent material (probably solid granuloma).
(b) Yellow tubercle (probably caseous granuloma).
(c) Empty TB lesion with a fibrotic wall and caseous center (probably fibrotic late-stage caseous granuloma).
(d) Partly liquefied and empty tubercle.
(e) Early-stage cell infiltration of lung tissue (probably nascent granuloma).

**Immunology of tuberculosis**

A multitude of interlinking cells and mechanisms are involved in the immune response to *M. tuberculosis* infection. The delicate balance between the bacteria and the host immune responses dictates whether the host response will lead to control of infection and possibly bacterial eradication, or whether infection will progress with increasing numbers of bacteria, lung tissue damage and transmission of *M. tuberculosis* organisms to new susceptible hosts (Saunders and Britton, 2007; Dorhoi et al., 2011).
The *M.tuberculosis* specific immune response comprises of different cell types ranging from T-cells to neutrophils, B-cells, and natural killer (NK) cells (Figure 4).

**Figure 4: The immune responses at different stages of the vicious cycle of TB.** Macrophages and dendritic cells (DCs) stimulate specific CD4+ and CD8+ T-cells, which are critical for protection against *M.tuberculosis*. CD4+ T cells are polarized into Th1 and Th17 cells. CD8+ T-cells contribute to protection by cytolytic mechanisms and IFN-γ production. Confounding factors include exogenous invaders (e.g., HIV, helminths) as well as endogenous cytokines (IL-4, IL-10, TGF-β) and inhibitory surface molecules (CTLA4, PD-1), which have the potential of disturbing the immune response. [Reproduced from Stefan HE Kaufmann. Immunity. 2010 (33): 567-577].

**CD4+ Th1-cells** are initially primed by *M.tuberculosis* antigens presented by MHC-class II molecules on the surface of professional phagocytes like DCs and macrophages present in the draining lymph nodes of the infected lung (Ladel *et al.*, 1995). The presentation of *M.tuberculosis* peptide-loaded MHC-class II complexes provides “signal 1” at the DC surface. Only in the presence of a second signal, which is provided by co-stimulatory molecules, such as CD80, CD86, CD40, CD27 and 4-1BB/CD137, and a third signal, provided by essential cytokines like IL-12, full activation and differentiation of Th1-cells takes place. In the absence
of any of these three essential signals, T-cell activation may either not be initiated, or result in T-cell tolerance/anergy. Th1-cells, which secrete IFNγ and tumor necrosis factor (TNF)-α as signature cytokines, are crucial for protective immunity against mycobacterial infections. Their induction is strictly dependent on the secretion of phagocyte-produced IL-12 (Ottenhoff et al., 2002).

Apart from the CD4+ Th1-cells, more recent evidence supports the prominent activation of Th17-cells in TB that produces IL-17A, IL-17F, in many cases IL-22 and TNF-α, and in some cases also IFNγ (Bettelli et al., 2008). Th17-cells are pro-inflammatory and mediate anti-microbial immunity against extracellular bacteria and fungi, particularly at mucosal surfaces. The exact mechanisms leading to Th17 activation and differentiation are less clearly understood than for Th1-cells but are believed to involve phagocyte-dependent production of IL-1β or IL-6, and transforming growth factor-beta (TGFβ) (Yang et al., 2008). Other studies have indicated the essential role of IL-23, a closely related family member of IL-12, in the induction and expansion of Th17-cells or Th17 memory cells (Sallusto and Lanzavecchia, 2009). IL17A has been found to be essential in forming mature granulomas in the lung following BCG or virulent M.tuberculosis infection (Okamoto Yoshida et al., 2010). It has been observed that multiple BCG re-vaccinations induce hyperactivity of Th17-cells. This has been detrimental on M.tuberculosis-infection due to increased immunopathology with IL17-MIP2-dependent influx of neutrophils and tissue destruction rather than containment of infection in the lung (Cruz et al., 2010). The major source of early IL-17 during infection, however, may not be CD4+ Th17-cells but rather T-cell receptor gamma delta (γδ) cells.

In addition to CD4+ T-cells, CD8+ T-cells also contribute to optimal immunity and protection against TB (Ladel et al., 1995). Although, the mechanisms underlying CD8+ T-cell activation in TB are incompletely defined, it is clear that DCs possess multiple pathways to load MHC-class I molecules. Firstly, active transmembrane transport, or leakage through micro-damaged membranes of M.tuberculosis phagosomal antigens to the classical cytosolic proteasome/MHC-class I presentation pathway, may lead to MHC-class I loading with M.tuberculosis peptides. Secondly, the suggestion that virulent mycobacteria can escape from the phagosome into the cytoplasm and thereby directly access the MHC-class I processing/presentation pathway may provide a new mechanism (Mazzaccaro et al., 1996; van der Wel et al., 2007). Thirdly, M.tuberculosis-infected cells can undergo apoptosis, leading to the formation of apoptotic vesicles that are taken up by DCs, after which the antigenic cargo is cross-presented through MHC-class I and class II molecules (Schaible et al., 2003). Fourthly, autophagy, which plays a prominent role in cellular homeostasis and in bacterial sequestration in vacuolar organelles, is involved in antigen presentation to, and cross-priming of, T-cells in
Chapter II

response to intracellular pathogens, including *M. tuberculosis* (Deretic, 2006). Thus, there are multiple probable pathways for activation of CD8+T-cells by phagosomal antigens.

CD8+ T-cells are endowed with multiple mechanisms to attack *M. tuberculosis*-infected cells. Importantly and in contrast to CD4+ Th1-cells, CD8+ T-cells are able to recognize non-phagocytic cells such as epithelial cells, which can also be infected by *M. tuberculosis* (Hernandez-Pando et al., 2000). In addition, all nucleated cells express MHC-class I molecules. Thus, CD8+ T-cells are able to survey larger numbers of cells and a broader range of cell types compared to CD4+ T-cells. CD8+ T-cells secrete granules that contain cytotoxic molecules such as perforin, granzymes, and granulysin which can lyse host cells and in the case of granulysin, also directly kill *M. tuberculosis* and other intracellular pathogens. In addition, CD8+ T-cells can induce apoptosis of infected target cells (Keane et al., 2000). CD8+ T-cells are also able to release Th1 cytokines such as IFNγ, TNFα, and in many cases also IL-2.

Besides T-cells, **NK-cells** and **B-cells** also play accessory roles in optimal immunity against TB (Maglione and Chan, 2009). Recent genome wide gene expression studies in TB patients revealed a striking neutrophil-associated expression pattern that was dominated by a type-1 IFN signaling signature. This profile was normalized after curative treatment of infection and had a core *M. tuberculosis*-specific component. Thus, other cells certainly contribute to host defense against *M. tuberculosis* in TB and may provide new biomarker signatures of pathogenesis, disease activity and protective immunity.

**Multi- or poly-functionality** is a term used to describe the simultaneous production of multiple cytokines (mostly IFNγ, IL-2, TNFα) or the expression of multiple effector functions (perforin, granulysin, cytolysis, etc.) by a single T-cell clone. Recently, expression pattern of three cytokines namely IL-2, TNFα and IFN-γ on a single cell basis has been proposed as an indicator of differentiation status of CD4 and CD8 T cells. However, continued antigenic stimulation can lead to a progressive loss of memory as well as effector potential, resulting in a pool of terminally differentiated CD4 or CD8 cells that only produce IFN-γ and are short-lived. CD4 cells that secrete IL-2, TNF-α or both can be sustained over a prolonged period of time and serve as a reservoir of memory CD4 T cells (T<sub>CM</sub>). The effector functions of these cells are guided by TNF-α which in conjunction with IFN-γ may result in the enhanced killing of *M. tuberculosis*. Cytokines like IL-2, although have little direct effector function, are crucial not only for promoting expansion of effector CD4 and CD8 T cells responses, but may also serve to program CD8 T cells for improved memory capacity and activate NK cell activity that could together contribute to an early control of infection following *M. tuberculosis* infection (Chan et al., 1992; Bloom et al., 1994; Wu et al., 2002; Stubbe et al., 2006; Williams et al., 2006; Seder et al.,
2008). Such observations clearly signify the importance of measurement of the immune responses based on multi-parametric analysis in comparison to the measurement of frequency of IFN-γ producing CD4 T cells alone (Kannanganat et al., 2007; Tilton et al., 2007; Hawkridge et al., 2008). However, the potential fate of these multi-functional CD4 T cells (T_{Efl}) that are characterized by the presence of IL-2, TNF-α and IFN-γ is governed by the amount of initial antigen exposure and/or innate immune factors. With higher stimulation, such as high dose of vaccination or infection, linear pathway of T cell development may not be evident and differentiation proceeds rapidly to IFN-γ secreting effector CD4 T cell populations (Foulds et al., 2008). Recent studies on this aspect have further emphasized the importance of measurement of more markers (like cell surface markers, CD44, CD62L) in determining the differentiation status and functionality of T cells in order to determine the quality and quantity of immune responses generated by vaccination, instead of relying on one or two signature cytokines (Junqueira-Kipnis et al., 2004; Seder et al., 2008; Henao-Tamayo et al., 2010).

Besides T-cells, endowed with protective effector functions, _M.tuberculosis_ also induces activation and expansion of regulatory T-cells (Treg) populations (CD25+FoxP3+), which suppress the host immune response to infection. These range from naturally occurring, broadly reactive CD4+ Tregs, to antigen-specific-CD4+ Tregs and newly identified CD8+ Tregs (Kursar et al., 2007; Joosten and Ottenhoff, 2008). These Tregs have multiple inhibitory effects. They can directly suppress CD4+ Th-cell activity through either the secretion of IL-10, CCL4 or the expression of TGFβ. They can also deactivate APCs and thus inhibit optimal priming of CD4+ responses. Moreover, they can inhibit the influx of CD4+ T-cells into draining lymph nodes, and/or inhibit their proliferation and expansion, thus delaying and inhibiting onset of adaptive immunity to _M.tuberculosis_ (Shafiani et al., 2010).

**Immunological biomarkers of tuberculosis**

The need for tuberculosis biomarkers arises, in part, from the lack of suitable tests to diagnose _M.tuberculosis_ or its products in host samples. The currently used diagnostic tests include; sputum smear test (by Ziehl-Neelsen or Auramine stain), chest X-rays, Mantoux test (by intradermal injection of tuberculin), histology (presence of caseating granulom), T cell IFNγ release assay (TIGRA). However, the diagnosis of tuberculosis continues to pose serious problems, mainly because of difficulties in differentiating between patients with active TB and those with healed lesions, normal BCG vaccinated individuals and unvaccinated Mantoux positives. The most widely used test involves microscopic detection of acid-fast bacilli in sputum (sputum smear test), which has a sensitivity of 34–80% (Davies and Pai, 2008).
Although the tuberculin test has aided in the diagnosis of tuberculosis for more than 85 years, its interpretation is difficult because sensitization with nontuberculous mycobacteria leads to false-positive tests. There have been numerous attempts to develop clinically useful serodiagnostic kits for tuberculosis but they have not proved to be clinically useful. A recently developed ex vivo \textit{M. tuberculosis} gene amplification test (GeneXpert MTB/RIF) can be used to diagnose TB and can also detect \textit{M. tuberculosis} strains resistant to rifampicin, one of the main antibiotics used in TB treatment, which serves as a marker for multidrug resistance. This test allows automated sample processing and produces results within two hours with excellent sensitivity (Boehme \textit{et al.}, 2010). However, its use is restricted to the identification of active pulmonary TB, as it cannot detect latent disease (Walzl \textit{et al.}, 2011).

Host biomarkers are therefore needed to help diagnose TB, to determine the response to therapy, to provide correlates of risk of TB and for correlates of protection against active disease. Currently, there are no sufficiently validated biomarkers to aid the evaluation of new TB vaccine candidates, for the improvement of TB diagnostics or for the development of more effective and shorter treatment regimens. Understanding the interplay between the host immune system and \textit{M. tuberculosis} may provide a platform for the identification of suitable biomarkers.

Exposure to \textit{M. tuberculosis} can result in different clinical outcomes, depending on the ability of the host innate and adaptive immune systems to control \textit{M. tuberculosis} (Figure 5). A wide range of specific and nonspecific host immune responses contribute to the differential outcomes of exposure and infection, although there is a lack of detailed understanding of the underlying mechanisms. The host response at different infection stages represents opportunities to measure individual markers or combinations of markers (biosignatures) that have diagnostic or prognostic potential (Walzl \textit{et al.}, 2011). During, initial phases of \textit{M. tuberculosis} infection i.e. during the \textbf{innate immune phase}, only 10% of the individuals develop immunological signs of infection; other 90% can eliminate the bacteria during the innate immune phase, without generating T cell memory [and thus have negative tuberculin skin test (TST) and IFN-γ release assay (IGRA) results]. In the \textbf{adaptive immune phase}, T cells are engaged by APCs, and this generates both effector memory T (T\textsubscript{EM}) cells and central memory T (T\textsubscript{CM}) cells. B cells are also activated and \textit{M. tuberculosis}-specific antibodies are produced. The infection may be cleared at this stage. However, most exposed individuals will enter the \textbf{quiescent phase}, in which \textit{M. tuberculosis} may persist for life within granulomas. Although the host fails to eradicate the pathogen, replication and dissemination of the bacteria are prevented. An optimal T\textsubscript{H} cell balance is required to control \textit{M. tuberculosis} while limiting immunopathology. This balanced reaction includes pro-inflammatory T\textsubscript{H}1-type responses.
characterized by IFN-γ, TNFα and interleukin-12 (IL-12) production) and TH17-type responses (characterized by IL-17 production). However, it also involves TH2-type responses (associated with IL-4 production) and regulatory T (T_{reg}) cell phenotypes that help in controlling excess of immunopathology. The **replicating phase** is symptomatic and at this stage the bacteria have escaped immune control. Granulomas are disrupted, the acute-phase response is activated and the levels of pro-inflammatory markers increase. Enhanced immunosuppression becomes evident as TH cell balance is disturbed and memory T cell populations and antibody production may change. HIV co-infection and associated CD4+ T cell depletion can trigger this immune escape by *M.tuberculosis*.

![Diagram of immune responses](image)

**Figure 5: Immune responses and potential host biomarkers of *M.tuberculosis* exposure and infection.** [Reproduced from Gerhard Walzl et al. Nature Reviews Immunology. 2011 (11): 343-354.]

**Animal models: Contributions in TB vaccine testing**

Animal models have played an important role in the testing of various anti-tubercular drugs and vaccine candidates. Though, each model has advantages and disadvantages, these models resemble one or the other facets of human disease. First and the foremost advantage of
using these models is that these animals can be easily infected by pulmonary route as a result of inhalation of a few virulent tubercle bacilli which get deposited in alveolar space in the same way as humans acquire infection. Further, it is easy to study various stages of TB progression like granuloma formation, liquefaction, cavity formation and haematogenous spread of the disease in animal models except mice (Caruso et al., 1999). The symptoms of the disease like fever, loss in weight, abnormal X-rays and respiratory distress can also be seen in these models. The animals eventually die of pulmonary insufficiency if left untreated, like the human patients. Because of these similarities in behavior the animal models and the humans in the susceptibility as well as resistance to TB, the animals are considered good models for evaluating new anti-TB vaccines as well as new anti-tubercular compounds.

Mouse is one of the most popular and economical experimental model that can be easily infected via aerosol with a low dose of bacilli (Rhoades et al., 1997). The major advantage of murine model for vaccine development lies in its ability to screen a large number of vaccines at a limited cost for mediating any protective effect (Gupta et al., 2007). The point which goes against using murine model is the nature of protection as the results may not be extrapolated directly to the human beings. The process of granuloma formation in mice after TB infection is altogether different from that seen in humans due to its innate resistance to TB. Granuloma in mice is characterized with the aggregation of lymphocytes towards center while in human and guinea pigs, lymphocytes form a peripheral ring with macrophages placed in the center (Ulrichs and Kaufmann, 2006) (Turner et al., 2003) (Kaplan et al., 2003). Mouse model has an edge in immunological studies of TB and hence found suitable for the first order screening of vaccines candidates and the efficacy of new candidate vaccines, and those showing good protection in mice are then evaluated in other animal models of TB.

Guinea pigs are extremely sensitive to infection with M.tuberculosis and can be infected with a small number of bacilli (Smith and Harding, 1977; Smith et al., 1991). The invariably fatal course of disease progression in this model provides a reliable parameter for identifying the effective anti-tuberculosis chemotherapy and vaccines (Dai et al., 1998) (Smith et al., 1991; McMurray, 2001). Guinea pig granuloma exhibits features similar to humans; (i) Guinea pigs develop classical granuloma which has Langhans multinucleated giant cells and (ii) with progression of active disease, the guinea pigs develop lung tissue necrosis, start losing weight and die due to the disease. A major difference from humans is the inherent susceptibility of guinea pigs as majority of the infected human beings can contain the TB infection. Though guinea pigs model of TB has several advantages, it suffers from some disadvantages which precluded its use as first order screening model. These are (i) high rearing cost of guinea pigs in a biosafety facility compared to low expenses involved in housing mice, and (ii) limited
availability of reagents to assess immunologic factors involved in imparting protection in vaccine studies.

**Rabbit** is being used as a model of human TB because of its innate resistance to TB as well as its close resemblance to human TB (Gupta and Katoch, 2009). The rabbit model offers certain advantages over murine and guinea pig models. First, when infected with *M. tuberculosis*, rabbits have a spectrum of disease that represents many of the specific stages of human disease. In general, rabbits are able to contain disease caused by virulent *M. tuberculosis*. Over time, the number of pulmonary bacilli declines and lesions regress (Lurie et al., 1952). Finally, rabbit granulomas, with their caseous centers, closely resemble the human granuloma. There are limited reports where rabbit model has been used for the evaluation of the candidate vaccines. The rabbit model like other animal models, has some limitations, including the paucity of commercial immunologic reagents. In addition, inbred rabbits in large numbers are not available, consequently experiments are carried out in outbred animals, giving rise to larger variations in results within each vaccination group.

**Non-human primate models** of TB are being used for many years for vaccine and drug testing studies (Barclay et al., 1973). Both Rhesus (*Macaca mulatta*) and cynomolgous (*Macaca fasicularis*) monkeys have been used. Rhesus macaques are highly susceptible to TB, the closely related cynomolgous macaque being more resistant. It may also be important that several of the host molecules implicated in TB infections are present in man and non human primates but are not present in mice and guinea pigs (Behar et al., 1999). The non-human primates have an edge over other models because of similarity to human TB, spectrum of disease and pathology as well as availability of the reagents for studying the immunological parameters. This translates into obtaining results that are more directly applicable to the human situation. However, these models have some disadvantages like high cost, biosafety facilities as well as non availability of inbred animals. Moreover, monkeys with TB are contagious to other animals, including other monkeys and the laboratory personnel posing a serious risk in an animal facility.

**Cattles** can be experimentally challenged which results in a reproducible disease and the trials can be completed within a relatively shorter time. The advantages of working with cattle model include the followings: (i) the experimentally induced disease is studied in the natural host with infections acquired predominantly via the respiratory route which helps in the meaningful screening of the vaccines, (ii) the disease has identical pathology in terms of granulomatous reactions and immune responses to that in humans, (iii) availability of a large number of immunological reagents, (iv) calves being immunologically competent at birth, neonatal vaccination is possible, (v) calves being sensitized to antigens of environmental
mycobacteria at younger age like humans, and (vi) BCG has variable efficacy in cattle like humans which provides an opportunity to detect better vaccines than BCG. The main disadvantages of this model are (i) instead of \textit{M.tuberculosis}, this model is based on the infection with \textit{M.bovis}, (ii) absence of cavitation like humans, and (iii) high cost involved in rearing the cattle. However, neonatal calves are a good model for testing vaccines in infants since they are immunocompetent at birth and become naturally sensitized to environmental mycobacteria at a younger age.

Although mice, guinea pigs and rabbits provide models for certain stages of TB infection and immunity, none of these animals transmit TB efficiently (Gupta and Katoch, 2009). For the optimal design and execution of such studies, as well as of studies on the effects of vaccination and other immunological interventions in preventing and promoting TB transmission, an investment in generating and validating immunological and genetic tools for use in these animals is necessary. Figure 6 depicts the various animal models that have been used to study individual stages of human TB.

\begin{figure}
\centering
\includegraphics{figure6.png}
\caption{Stages of the immunological life cycle of human TB that can currently be modelled in experimental animals. Shown are the animal models (zebrafish, mouse, guinea pig, rabbit, cattle and non-human primate) that have been used to study individual stages of the immunological life cycle. \cite{Ernst2012}}
\end{figure}
**Fight against Tuberculosis: A daunting task**

*A brief history of vaccines*

The notion of protective immunity can be traced back to the observation in the fifth century that individuals who had recovered from disease during the Plague of Athens were protected from subsequent attacks. However, the birth of the science of immunology is most readily attributed to the demonstration by Edward Jenner at the end of the 18th century when individuals intentionally inoculated with material from cowpox-infected cattle were protected from smallpox. This demonstration predated evidence for the microbial origin of infectious diseases obtained by Robert Koch and Louis Pasteur. It also predated the elucidation of the immunological factors underlying this protective effect by Von Behring and many others. These “immunologists” went on to develop this field as a discipline and to illuminate the crucial role of immunity and inflammation in infectious diseases and in many other aspects of human physiology (Allen et al., 1999). Over the years, the fields of immunology and clinical vaccinology has diverged: immunology became progressively focused on model systems that allowed its intricacies to be probed in cellular and molecular detail, whereas vaccinology addressed more practical problems, focusing on humans and other species for which vaccines were intended. Nonetheless, vaccinology has been a stunning success, with vaccination being one of the greatest public health measures of the past century, and arguably the most economical medical intervention (Centers for Disease Control and Prevention, 1999). The eradication of smallpox in 1977 is a landmark achievement. The potential for eradication of polio is at hand, although both public health and immunobiological challenges remain (Serazin et al., 2010).

Early in 1921, hopes were that TB could be conquered by vaccination with the newly developed *M.bovis* BCG vaccine named after its discoverer Calmette and Guérin in Lille, France. These hopes were further boosted by the development of first anti-tubercular drug during World War II by Selman Waksman who discovered that streptomycin was bacteriostatic for *M.tuberculosis*. Initially treatment with streptomycin appeared highly efficacious but the tide turned when drug resistance rapidly developed. Despite this early writing on the wall, the conception that TB could be conquered by antibiotics and BCG vaccination led to complacency for several decades.

*The Bacille Calmette-Guérin (BCG) vaccine: The scientific fable and the lessons learned*

BCG is widely used and it is effective in preventing severe forms of TB, mostly disseminating and meningeal forms in children (Hart and Sutherland, 1977; Bloom, 1994).
BCG has been safe and a part of the Expanded Program on Immunization (EPI) since the early 1970s and has been administered over 4 billion times globally (Kaufmann, 2010). BCG also reduces the occurrence of other mycobacterial diseases. However, BCG fails to prevent pulmonary TB among adults and adolescents worldwide. Moreover, BCG does not protect against latency, reactivation or reinfection TB. Although BCG vaccine is the world’s most widely used vaccine against tuberculosis, ironically, it is also considered to be one of the most controversial vaccines (Fine, 1995). Estimates of protection imparted by BCG against pulmonary TB is inadequate and vary greatly from 0-80% (Antas and Castello-Branco, 2008) (Milstien and Gibson, 1990; Lugosi, 1992; Colditz et al., 1995). For example, BCG vaccination in British school children, in 1952, showed 77% to 84% efficacy, whereas in the trial conducted in Chingleput, India, the protective efficacy was nil against pulmonary TB in adults. This variability has been attributed to genetic variability among BCG strains, rapid clearance of BCG in some populations, defects in its preparation, and also to environmental influences such as its exposure to sunlight, poor maintenance of cold chain, genetic or nutritional differences amongst populations, or exposure to environmental mycobacterial species. Another explanation to account for the variable results in the trials of vaccine-efficacy could be a progressive over-attenuation of BCG during prolonged passage through the laboratory. It is still unclear why the protective effect of neonatal or early age BCG vaccination often begins to wane in early adolescence. In other words, almost 90 years after the first administration of BCG to a baby and more than 100 years since its development, we don’t even understand the precise immune mechanisms that is responsible for protection in BCG vaccinated infants (Fine, 1995; Kaufmann, 2010). Besides, implementation of BCG vaccination faces problems (Harding and Smith, 1977). Firstly, vaccination with BCG induces a delayed type hypersensitivity (DTH) skin response that cannot be distinguished from infection with M.tuberculosis and therefore it compromises the use of purified protein derivative (PPD) of M.tuberculosis (tuberculin) in skin tests for diagnostic or epidemiological applications. Secondly, BCG is also contraindicated for its use in immunocompromised patients and newborns with HIV resulting in the increased risk of developing disseminated BCG-osis (Hesseling et al., 2007) (Clements et al., 1987) (Ninane et al., 1988; Quinn, 1989).

Expectations from a new TB vaccine

The shortcomings of BCG vaccine have provided the impetus for making efforts to identify alternative vaccines for TB. One approach exploited is the identification of M.tuberculosis specific antigens with potentials for inducing protective immunity and further
evaluation of such antigens in animal models of TB before conducting large-scale efficacy trials in humans. The world needs new vaccines to replace or improve BCG. Expectation from a new vaccine is to “perform better than BCG” by (i) preventing establishment of initial infection, (ii), preventing disease progression by controlling bacillary multiplication and dissemination, (iii), preventing pathological damage to the host and (iv), conferring long lasting and sustained protection. A truly effective TB vaccine may, therefore, have to elicit an immune response that is greater than that induced by natural infection and should fair well in waving off the limitations of BCG. In addition, various different populations including those vaccinated with BCG, adult population and those infected with M. tuberculosis or with HIV have to be protected.

Problems associated with the development of TB vaccines

Unfortunately, the development of efficacious vaccines against TB presents diverse and complex challenges:

- **Complexity of TB disease and heterogeneity of the host response to infection**: TB is a complex infectious disease with multi-faceted stages in pathogenesis. Understanding the host immunity to TB is very important. The host factors that determine why some individuals are protected from infection while others go on to develop disease are unclear. Genetic factors of the host and of the pathogen itself may be associated with an increased risk of patients developing the active TB. Only through a thorough knowledge of how M. tuberculosis is recognized and controlled by the immune system of different individuals, it is possible to design and evaluate new vaccine candidates against various populations; infants never exposed to TB, adults who have been exposed, those who are already infected and HIV infected individuals.

- **Ambiguous nature of protective immunity against TB**: A much better understanding of the nature of protective immunity that is generated against TB is needed. This will aid to develop better vaccine than BCG and to minimize the likelihood of vaccine-induced pathogenic immune responses and the risk of adverse effects of new TB vaccines.

- **Absence of reliable surrogate TB biomarkers**: The identification of protective biomarker signatures would greatly facilitate vaccine discovery and testing. If it was possible to identify a correlate for vaccine induced protection especially in humans, discovery of high profile TB vaccine candidates and clinical testing could be markedly accelerated.

- **Unexplained latent M. tuberculosis infection and the problem of reactivation TB**: One third of population is latently infected with M. tuberculosis. Latent M. tuberculosis infection can
reactivate years later and infection does not guarantee resistance to a subsequent second infection. It is very crucial to understand the mechanisms responsible for the development of latency in order to design any new vaccine that does not allow establishment of latency and thus eliminate any probability of reactivation.

**Various experimental animal models:** Arguably, animal models play an important role in predicting the outcome of a vaccine against human diseases. In the case of TB, several animal models such as mice, guinea pigs and non primates are available which can be used to evaluate the protective efficacy of a TB vaccine candidate. However, in spite of their merits and caveats, no animal model can correctly predict the efficacy of a TB vaccine in humans.

**Efforts towards TB vaccine development**

Due to several important unanswered questions, we do not have a perfect approach to produce a perfect TB vaccine. However, based on the available knowledge and technology, TB investigators have made tremendous efforts in the last two decades to develop new TB vaccines and evaluate them in the existing animal models so that some of these can be channeled for human clinical trials. In the absence of a verifiable strategy, the empirical approaches towards the development of TB vaccines are driven by scientific optimism and epidemiological dividends that an efficient TB vaccine can potentially offer. As a result, several TB vaccines have been developed, many are already progressing through clinical studies and we have a reasonably robust pipeline of TB vaccines.

1. **Modified BCG vaccines**

The main rationale for recombinant BCG (rBCG) development is the hypothesis that the immunogenicity of BCG has been weakened by continuing attenuation and gene loss during several *in vitro* passages (Behr and Small, 1997). Thus, adding deleted genes back into BCG or increasing the expression of selected protective antigens might improve the effect of BCG vaccination (Nasser Eddine and Kaufmann, 2005; Hernandez-Pando *et al.*, 2007). rBCG30 over-expressing antigen 85B has resulted in improved protection against TB in guinea pigs and is immunogenic in humans (Hoft *et al.*, 2008; Tullius *et al.*, 2008). This vaccine has successfully passed phase I clinical safety trial but is currently on hold. Recently, an rBCG strain over-expressing Ag85A, Ag85B, and TB10.4 (Sun *et al.*, 2009) was reported to be safe and induced high levels of IFNY producing T-cells in mice. However, its protective efficacy against TB was not improved substantially over wild-type BCG. Ryan *et al.* evaluated an rBCG
strain over-expressing a murine monocyte chemotactic protein 3 (BCG-MCP-3) (Ryan et al., 2007). Although BCG-MCP-3 was less virulent in the lungs and spleens of infected mice, it provided a protection comparable with the BCG in C57BL/6 mice infected with M.tuberculosis H37Rv. RAG-/- mice infected with BCG-MCP-3 survived significantly longer than the BCG controls, demonstrating the safety of this vaccine. Grode et al. demonstrated that mice immunized intravenously with listeriolysin secreting rBCG vaccine exhibited greater protection than BCG, as assessed by lung and spleen bacillary loads (Grode et al., 2005). Furthermore, a urease C-deficient Hly+ rBCG vaccine provided better protection than the urease-containing strain. In addition, in vitro studies suggest that ΔureC Hly+ rBCG promotes antigen translocation into the cytoplasm and enhanced apoptosis of the host cell facilitating cross-priming by DCs, inducing strong CD4 + and CD8 + T-cell responses (Desel et al., 2011). In addition, this strain was found to activate Th17-cells, which probably facilitated optimal immunity against M.tuberculosis (Desel et al., 2011). Recently, rBCG85C over expressing M.tuberculosis antigen 85C (developed in our laboratory), was evaluated for its protective efficacy in guinea pigs against M.tuberculosis challenge by aerosol route. Immunization with rBCG85C resulted in a substantial reduction in the lung and spleen bacillary load with a commensurate reduction in pathological damage, when compared with the animals immunized with the parent BCG strain (Jain et al., 2008).

2. Attenuated M.tuberculosis vaccines

Whole genome microarray analysis revealed the absence of 15-16 regions that have been deleted from the genome of BCG, but are present in M.tuberculosis. These so-called Regions of Difference (RD) are believed to encode at least 129 open reading frames, which include several regulatory genes (Mahairas et al., 1996; Behr and Small, 1997). These missing regions may encode potential antigenic determinants that could increase the immunogenicity of a vaccine. This makes the use of attenuated M.tuberculosis strains rather than BCG, for the generation of appropriate immune responses, an attractive idea (Hernandez Pando et al., 2006). One of the studies involving attenuated M.tuberculosis strain employed subcutaneous immunization with the ΔRD1ΔpanCD (mc26030) mutant which contains deletions in the major M.tuberculosis RD1 virulence gene cluster as well as in the biosynthetic pathway of pantothenate (Sambandamurthy et al., 2006). This strain was safe and induced efficient protection in both immunocompetent and immunocompromised mice. However, in various studies, results partially depended upon the animal model being used. Mice vaccinated with the ΔsecA2 mutant (secA2 deletion mutant of M.tuberculosis) had significantly lower lung and spleen bacillary counts and longer survival times when compared with the BCG-vaccinated group.
(Hinchey et al., 2007). However, the same vaccine when administered to guinea pigs, reduced lung and spleen bacillary loads no more effectively than vaccination with BCG. Another promising attenuated \textit{M. tuberculosis} strain is SO2 strain (\textit{phoP} deletion mutant of \textit{M. tuberculosis}) which exhibited similar level of protection in mice, although guinea pigs vaccinated with SO2 exhibited significantly increased survival time when compared with BCG (Martin et al., 2006).

3. **DNA vaccines**

DNA vaccination is one of the most commonly used approaches employed to evaluate the protective efficacy of an antigen in animal models. This may be due to the relative ease of preparation of DNA vaccines, which avoids complicated protein purification and chemical adjuvant issues. DNA vaccines are also amenable to the insertion of biological adjuvants, such as CpG motifs or cytokine genes. The plasmid DNA used in DNA vaccination may directly transfected stromal cells (such as muscle cells) or dendritic cells (DCs). In these cells, a cytosolic DNA receptor that has not yet been identified induces the activation of TANK-binding kinase 1 (TBK1) and IκB kinase-ε (IKKe) through the stimulator of IFN genes (STING), leading to the activation of interferon-regulatory factor 3 (IRF3) and resulting in the production of type I IFNs. The antigens encoded by the transfected plasmid DNA can also be expressed in stromal cells and DCs. In DCs, these antigens may be directly processed and presented on MHC class I molecules to naive CD8+ T cells. Alternatively, antigens may be indirectly acquired by DCs from stromal cells and then cross-presented to CD8+ T cells or presented to naive CD4+ T cells on MHC class II molecules (Huygen et al., 1996; Tascon et al., 1996; Zhu et al., 2005; Huygen, 2006). Type I IFN expression by stromal cells and DCs seems to be important for promoting the cross-presentation activity of DCs, as well as for the differentiation of Th cells and the promotion of Th1-type isotype switching in B cells (Figure 7).

Several investigators are pursuing the development of prophylactic DNA vaccines for TB (Lowrie, 2006). DNA vaccine consisting of a cocktail of mycobacterial antigens, Ag85B, MPT64 and MPT83 along with the IL-12 gene as an adjuvant exhibited a significantly reduced lung CFU in mice in the DNA-vaccinated group when compared with the BCG-vaccinated group (Yu et al., 2007). Derrick et al. evaluated the protective efficacy of a DNA vaccine (pE6/85) expressing an ESAT-Ag85B fusion protein in mice (Derrick et al., 2004). The authors reported no significant difference in bacillary loads in the lungs and spleens of mice given pE6/85 as a primer or booster to BCG, but bacillary loads were significantly reduced when compared with mice given BCG alone. Furthermore, if the mice were challenged at 1 year post-primary
immunization, bacillary loads in the lungs were significantly reduced when the pE6/85 fusion protein was given as a booster to BCG.

Moreover, several candidate DNA vaccines have been evaluated as adjunct to chemotherapy for their ability to enhance the efficacy of the treatment (Lowrie and Silva, 2000; Li and Zhu, 2006). These efforts have resulted in varying degree of success (Dutt and Khuller, 2001; Li and Zhu, 2006; Nuernberger et al., 2006; Aagaard et al., 2011; Coler et al., 2012; Gupta et al., 2012a). The use of hsp65 based DNA vaccine in immunotherapeutic mode after completion of chemotherapy has been shown to effectively prevent the reactivation of TB in mice (Lowrie et al., 1999). Moreover, hsp65 based DNA vaccine has also been shown to be a valuable adjunct to antimycobacterial chemotherapy which not only accelerates the treatment
against active TB cases but also improves the treatment of MDR TB (Silva et al., 2004). Similarly, the use of Ag85A DNA vaccine either alone or in combination with PstS-3 DNA vaccine as an adjunct to chemotherapy has been shown to markedly prevent reactivation and reinfection of TB besides providing effective treatment of MDR TB in mice (Ha et al., 2003; Ha et al., 2005; Liang et al., 2011). In addition, immunotherapy with a combination of DNA vaccines expressing Ag85B, MPT-64 and MPT-83 along with chemotherapy has been shown to significantly reduce the duration of chemotherapy (Zhu et al., 2005). By virtue of their ability to enhance therapeutic efficacy, to reduce the magnitude of disease transmission and to bring down the reactivation of latent TB infections, the importance of DNA vaccines in the control of the disease can not be overemphasized.

4. Subunit vaccines

Subunit TB vaccines based upon purified mycobacterial proteins and peptides have been the focus of many vaccine studies (Andersen and Doherty, 2005b). Subunit vaccines in TB are mostly based on recombinant proteins admixed with proper adjuvants, or the use of attenuated viral vectors. Although subunit vaccines theoretically could be used as priming vaccines, current views are that they may be mostly used as booster vaccines to enhance the immunity of BCG-, recombinant BCG-, or attenuated M. tuberculosis-priming vaccines. Therefore, boosting with the subunit vaccines is anticipated to result in strong, long-lived immune responses in already primed individuals (Lindblad et al., 1997; Andersen and Doherty, 2005a; Ottenhoff and Kaufmann, 2012). Progress has been made in the development of chemical adjuvants that would drive a strong cell-mediated immune response to mycobacterial proteins or peptides and be approved for use in humans. Various combinations of monophosphoryl lipid A (MPL) and dimethyldioctadecylammonium bromide (DDA) have been used by several investigators (e.g., IC31, AS2 and LKT63) (Ly and McMurray, 2008; Rappuoli et al., 2011) (Ly and McMurray, 2008). These adjuvants induce Th1 bias immune responses. In a novel attempt to improve the effects of subunit vaccination by selectively stimulating Toll-like receptors (TLRs), Wang et al. created a fusion protein vaccine consisting of ESAT-6 fused to the putative TLR 2 agonist, Rv1411, known as CSU-F36 in a DDA adjuvant, with or without MPL (Wang et al., 2007). The authors observed approximately the same degree of protection in the CSU-F36 and BCG-immunized mice with improved histopathological appearance. Jeon and colleagues prepared an aqueous fraction of Triton X-100-soluble cell wall proteins from M. tuberculosis and mixed it with incomplete Freund’s adjuvant and reported that the bacillary loads in the lungs and spleen were comparable in mice vaccinated with BCG or their subunit vaccine (Jeon et al., 2008). Moreover a subunit vaccine composed of PE_PGRS (Rv1759c) has
Review of literature

been reported to prevent reactivation in a mouse model of chronic infection (Campuzano et al., 2007). Some of the TB vaccines currently in Phase I clinical trials are subunit vaccines indicating the success of this vaccine-development strategy.

5. **Heterologous prime-boost vaccination strategies**

The fact that the immune system once primed with an antigen elicits a heightened immune response to the secondary exposure of the antigen has resulted in effective prime boost vaccination strategies against TB. Repeated administration with the same vaccine called as **homologous boosting** has proved to be relatively inefficient at boosting cellular immunity, instead it generates a very strong humoral response. **Heterologous boosting** on the other hand involves sequential administration of vaccines with appropriate intervals by using different antigen-delivery systems such that the immune system is primed with the antigen by using one vector and is then boosted with the same antigen delivered through a different vector. This approach induces a greater repertoire of antigen specific T cells and a selective enrichment of high avidity T cells than that achieved by homologous boosting.

There are three major types of vaccine strategies that can be used as a booster: protein-based, plasmid DNA-based and viral-based.

**Protein based:** One of the most advanced protein based vaccine is the Hybrid 1 fusion protein, which consists of Ag85B fused to ESAT6. When administered together with the potent IC31 adjuvant, it results in strong CD4+ Th1 IFNγ induction in humans (Ottenhoff et al., 2010). This was first reported in naive human volunteers (van Dissel et al., 2010). An encouraging observation was the long-lasting high-level immune response detectable 2.5 years after the last vaccination. A very similar fusion protein, Hyvac 4, which consists of Ag85B fused to the antigen TB10.4 is in a parallel clinical development program. A similar approach was followed by GlaxoSmithKline (GSK) that designed the M72 fusion protein, consisting of *M.tuberculosis* antigens Rv1196 and Rv0125 (Von Eschen et al., 2009). The M72 fusion protein was admixed with different GSK synthetic adjuvants and had a favorable clinical profile in terms of safety and immunogenicity. The currently favored adjuvant is the AS01E, which contains mono-phosphoryl lipid A mixed with QS21. This vaccine is currently undergoing phase IIa trial. Other antigens close to phase I clinical trial include heparin-binding hemagglutinin (HBHA) (Rouanet et al., 2009). HBHA is expressed by both *M.tuberculosis* and BCG and is heavily methylated at its C-terminal portion, against which T-cell responses seem to be specifically directed (Locht et al., 2006).

**Plasmid DNA based:** DNA vaccines have emerged as an important advancement in the field of vaccine discovery that are being used as a prophylactic vaccine as well as to boost the
immunity imparted by BCG. DNA vaccine expressing α-crystallin- a key latency antigen of *M. tuberculosis* has been recently evaluated as a booster to BCG or recombinant BCG expressing the same antigen. Both ‘BCG prime – DNA boost’ regimen (B/D) and “rBCG prime – DNA boost” strategy (R/D) confer robust protection in guinea pigs along with a reduced pathology in comparison to BCG vaccination in both lungs and spleen (Dey *et al.*, 2011a; Dey *et al.*, 2011b). In addition, both the regimens also conferred enhanced protection in mice.

**Viral based:** Apart from the recombinant proteins and DNA vaccines, which have been recommended as boosting agents, viral vectors are now being highly used to boost the immunity imparted by BCG. Two viral delivery systems for TB antigens are currently in clinical trials: Modified Vaccinia virus Ankara (MVA) based and adenoviral based delivery vehicles. MVA expressing Ag85A has proved to be extremely immunogenic in both naive as well as BCG-primed individuals, inducing high Ag85A-specific CD4+ T cell responses in humans (McShane *et al.*, 2004; Scriba *et al.*, 2010). According to a recently conducted infant phase IIb “Proof-of concept” (PoC) trial in South Africa, MVA85A appears to be safe and well tolerated, confirming similar findings from previous phase I and phase IIa clinical trials using this vaccine. However, vaccine efficacy analysis was based on the number of TB cases amongst the vaccinated versus control subjects which was not statistically significant. Moreover, there was no evidence of protection against *M. tuberculosis* infection. Another viral vector is replication-deficient adenoviral systems which have either used Ad5 or Ad35 platforms. Ad35 carrying *M. tuberculosis* antigens; Ag85A, Ag85B, and TB10.4 was highly immunogenic in humans, inducing strong CD4+ and CD8+ T cells and IFNγ responses (Radosovic *et al.*, 2007; Abel *et al.*, 2010).

### 6. Atypical mycobacterial vaccines

Prior exposure to various mycobacteria can potentially alter immune responses in a subsequent infection with *M. tuberculosis*. Mycobacterial strains which share cross-reactive antigens with *M. tuberculosis* have also been considered as alternatives to BCG. In a human trial, which involved ~10,000 adolescents, *M. microti* proved to be safe and was found to protect with the same efficacy as BCG, averaging 77% over a 20-year follow-up period (Hart and Sutherland, 1977; Manabe *et al.*, 2002; Brodin *et al.*, 2004). Several studies have also been carried out with killed/live or recombinant *M. vaccae* as both prophylactic and immunotherapeutic agent resulting in protective efficacy similar to BCG (Etemadi *et al.*, 1992; Stanford and Stanford, 1994; Onyebujoh *et al.*, 1995). *M. vaccae* given as a single injection to
patients within the first few weeks of starting chemotherapy for pulmonary tuberculosis appeared to improve the cure rate and reduce the number of deaths (Stanford and Stanford, 1994). In addition, there are reports that state that mice vaccinated with *M. habana* were protected from challenge with *M. tuberculosis* in both immunocompetent and immunocompromised states (Chaturvedi *et al.*, 1999; Prem Raj *et al.*, 2003). In another approach, immunization with *Mycobacterium indicus pranii* (*MIP;* formerly called as *Mu*) imparts significantly better protection both in mice and guinea pigs when compared with BCG against TB (Gupta *et al.*, 2012b). As an immunomodulator, when evaluated as an adjunct to chemotherapy, *MIP* resulted in a greater reduction in the bacterial load in both lungs and spleen (Faujdar *et al.*, 2011; Gupta *et al.*, 2012a). Based on its demonstrated immunomodulatory action in various human diseases, *MIP* is the focus of several clinical trials and successful completion of one such trial has resulted in its use as an immunotherapeutic vaccine ‘Immuvac’ against leprosy (Nath, 1998). Moreover, recently, the complete *MIP* genome has been sequenced that may help us in understanding the mechanism of protection mediated by this immunomodulator (Saini *et al.*, 2012).

**Global status of TB vaccine development: Vaccines in the pipeline.**

According to “Stop TB Partnership Working Group on New TB Vaccines”, there are more than a dozen TB vaccine candidates that have entered clinical trials in 2011, and many more are in the pre-clinical pipeline to be considered for testing in phase I clinical trials (2011).

1. **Candidates Tested in Clinical Trials:** At present, the most promising TB vaccine candidates that are in clinical pipeline are listed in Table 1. These include:
   a) **Pre-exposure prime vaccination:** VPM 1002.
   b) **Pre-exposure boost vaccination:** Crucell Ad35/ Aeras-402, AdAg85A, Hybrid 1 + IC31, Hybrid 56 + IC31, Hybrid 1 + CAF01, M72 + AS01 or ASO1, Aeras-404: HyVac + IC31.
   c) **Therapeutic vaccinations:** RUTI, MIP (*M. indicus pranii*)

2. **Candidates in Preclinical Studies & GMP:** Besides the candidates in clinical trials pipeline, there are 5 TB vaccine candidates (as of December 2011) that are not yet in clinical trials but had been manufactured under good manufacturing practice (GMP) for clinical use. These candidate vaccines had undergone some preclinical testing that met regulatory standards. These include *M. tuberculosis* ΔleuCDΔpanCDΔsecA2, MTBVAC [ΔphoP, ΔfadD26], HBHA, HG85 A/B and *Hsp* DNA vaccine.
3. **Next Generation Candidates:** These include several TB vaccine candidates (~34 candidates) that are in the research and development stage with some preclinical testing performed to show that they may confer protection. Some of them are: IKEPLUS, rBCG(mbt)30, rBCG38, BCGΔsapM, rBCG85C, BCG prime-DNAcr boost and rBCGacr prime-DNAcr boost etc. (last three are from our laboratory).

**Table 1.** Most advanced TB vaccine candidates in clinical trials.

<table>
<thead>
<tr>
<th>Type</th>
<th>Candidate</th>
<th>Description</th>
<th>Clinical Trial Status</th>
<th>Institution/Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-exposure prime vaccination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recombinant BCG</td>
<td>VPM 1002</td>
<td>rBCG-expressing listeriolysin and urease deletion</td>
<td>Phase Ila ongoing</td>
<td>Max Planck Institute for infection Biology/ Vakzine Projekt Management</td>
</tr>
<tr>
<td></td>
<td>rBCG30*</td>
<td>rBCG-expressing Ag85B</td>
<td>Phase I completed/on hold</td>
<td>UCLA school of Medicine/Aeras/NIH/NIAID</td>
</tr>
<tr>
<td></td>
<td>Aeras-422*</td>
<td>rBCG-expressing perfringolysin and Ag85A, 85B, Rv3407</td>
<td>Phase I terminated due to side effects</td>
<td>Aeras</td>
</tr>
<tr>
<td><strong>Pre-exposure booster vaccination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral-vector</td>
<td>Oxford MVA85A/Aeras-485*</td>
<td>Modified Vaccinia Ankara-expressing Ag85A</td>
<td>Phase Iib completed</td>
<td>Oxford Emergent Tuberculosis Consortium (OETC), Aeras</td>
</tr>
<tr>
<td></td>
<td>Crucell Ad35/Aeras-402</td>
<td>Replication-deficient adenovirus 35-expressing Ag85A, Ag85B, TB10.4</td>
<td>Phase Iib ongoing</td>
<td>Crucell/Aeras</td>
</tr>
<tr>
<td></td>
<td>AdAg85A</td>
<td>Replication-deficient adenovirus 5-expressing Ag85A</td>
<td>Phase I</td>
<td>McMaster University</td>
</tr>
<tr>
<td><strong>Fusion protein in adjuvant</strong></td>
<td>M72+AS01 or AS01</td>
<td>Fusion of Rv1196 and Rv0125 in adjuvant AS01 or AS02</td>
<td>Phase Ila ongoing</td>
<td>GlaxoSmithKline/ Aeras</td>
</tr>
<tr>
<td></td>
<td>Hybrid 1+IC31</td>
<td>Fusion of Ag85B and ESAT-6 in adjuvant IC31</td>
<td>Phase I, soon entering Ila</td>
<td>Statens Serum Institut/TBVI</td>
</tr>
<tr>
<td></td>
<td>Hybrid 56+IC31</td>
<td>Fusion of Ag85B, ESAT-6 and Rv2660c in adjuvant IC31</td>
<td>Phase I ongoing</td>
<td>Statens Serum Institut</td>
</tr>
<tr>
<td></td>
<td>Hybrid 1+CAF01</td>
<td>Fusion of Ag85B and ESAT-6 in adjuvant CAF01</td>
<td>Phase I ongoing</td>
<td>Statens Serum Institut</td>
</tr>
<tr>
<td></td>
<td>Aeras-404; HyVac+IC31</td>
<td>Fusion of Ag85B and TB10.4 in adjuvant IC31</td>
<td>Phase I</td>
<td>Statens Serum Institut/Tunis, Pasteur/Aeras/Intercell</td>
</tr>
<tr>
<td><strong>Therapeutic vaccination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole bacterial vaccine</td>
<td>MIP (M.indicus pranii)</td>
<td>Whole cell saprophytic non-TB mycobacterium</td>
<td>Phase III</td>
<td>DBT India/ M/s. Cadila Pharmaceuticals Ltd.</td>
</tr>
<tr>
<td></td>
<td>RUTI</td>
<td>Detoxified <em>M.tuberculosis</em> in liposomes</td>
<td>Phase Ila ongoing</td>
<td>Germans Trias i Pujol Hospital, Archivel Farma S.L.</td>
</tr>
<tr>
<td></td>
<td>M.vaccae V7*</td>
<td>Inactivated <em>M.vaccae</em></td>
<td>Phase III completed</td>
<td>NIH, immunomodulation</td>
</tr>
<tr>
<td></td>
<td>M.smegmatis*</td>
<td>Whole cell extract</td>
<td>Phase I completed</td>
<td></td>
</tr>
</tbody>
</table>

*, Vaccine candidates that have been in clinical trials in the past, but are not currently being tested.

In summary, there is a reason for optimism in the field of TB vaccines that: there is a substantial portfolio of interesting TB vaccine candidates in clinical phase-1/II testing, some of which are already fairly advanced in the TB vaccine pipeline. This is complemented by numerous promising candidates further upstream in the pipeline, however our efforts must be directed to keep this pipeline full of TB vaccine candidates.

**Development of pre-exposure vaccination strategy**

Until now virtually all TB vaccine strategies have focused on pre-exposure vaccination, which ideally should induce strong, long-lasting memory T-cell responses that are rapidly mobilized after infection with *M.tuberculosis* and mediate protection. Recent work has revealed that *M.tuberculosis* alters its metabolic state from actively replicating to slow or non-replicating state during infection. This is accompanied by significant changes in *M.tuberculosis* gene expression profile that also have an impact on which antigens are available to the immune system. *M.tuberculosis* genome comprises of ~4000 genes, which are differentially expressed under various conditions (Cole *et al.*, 1998). A clear understanding of specific sets of antigens that are expressed during different stages of disease such as, early infection, active disease, latency or reactivation and their immunological characterization are keys to the development of effective prophylactic or post-exposure vaccines.

In this study, by deleting the function of three virulence genes, namely, *mptpA* (Rv2234), *mptpB* (Rv0153c) and *sapM* (Rv3310), we have developed an *M.tuberculosis* mutant, MtbdΔmms and evaluated its protective efficacy in guinea pigs against TB. The importance of these genes is described below.

1. **Mycobacterial secretory acid phosphatase (*sapM, Rv3310*)**

   SapM was first identified in culture filtrate of *M.tuberculosis* as a secreted acid phosphatase (Saleh and Belisle, 2000). SapM was initially found to exhibit activity against phosphoenolpyruvate, glycerophosphate, GTP, NADPH, phosphotyrosine and trehalose-6-phosphate as substrates (Saleh and Belisle, 2000). In a later study, Vergne *et al.* showed that PI3P, a membrane trafficking regulatory lipid that is essential for phagosomes to acquire lysosomal constituents, is retained on phagosomes containing dead mycobacteria but is continuously eliminated from phagosomes with live bacilli (Vergne *et al.*, 2005). SapM was found to possess PI3P phosphatase activity and was responsible for PI3P removal (Vergne *et al.*, 2005). This suggested that SapM mediates the arrest of phagosome maturation by disrupting
the recruitment of PI3P effector proteins such as early endosome antigen 1 (EEA1) (Figure 8). Moreover, addition of SapM to an in vitro assay inhibited phagosome fusion with late endosomes (Vergne et al., 2005). Deletion of sapM from BCG resulted in a vaccine strain characterized by longer survival of mice lethally challenged with M. tuberculosis (Festjens et al., 2011). The increased efficacy of the vaccine was accredited to the efficient activation and recruitment of dendritic cells to the draining lymph nodes in the absence of SapM, thus allowing successful antigen presentation and activation of the adaptive immunity by dendritic cells (Festjens et al., 2011). A recent study showed that the fbpA/sapM double mutant of M. tuberculosis was attenuated for growth and was more immunogenic in macrophages as compared to M. tuberculosis (Saikolappan et al., 2012). Hence, sapM was selected as a potential gene to be deleted in M. tuberculosis.

Figure 8. Role of M. tuberculosis phosphatases in host pathogen interaction. [Reproduced from D. Wong. Trends in Microbiology. 2013 (2):100-109.]
2. **Mycobacterial protein tyrosine phosphatase A (mptpA, Rv2234)**

MptpA is secreted by *M. tuberculosis* into the host macrophage cytosol and disrupts key components of the endocytic pathway, resulting in the arrest of phagosome maturation (Bach *et al.*, 2008; Wong *et al.*, 2011). Human vacuolar protein sorting 33B (VPS33B) was identified as the cognate substrate of MptpA by using a substrate-trapping approach (Figure 8). Dephosphorylation of VPS33B by MptpA inactivates the host protein, leading to the inhibition of phagosome-lysosome fusion by inhibiting V-ATPase trafficking to the mycobacterial phagosome (Koul *et al.*, 2000; Bach *et al.*, 2008; Wong *et al.*, 2011). Interestingly, MptpA binding to the V-ATPase is a prerequisite for the dephosphorylation of VPS33B within the macrophage (Wong *et al.*, 2011). This suggests that MptpA binding to V-ATPase localizes MptpA to the vicinity of its substrate within the host cytosol. Thus, it is possible that MptpA binding to the V-ATPase might also disrupt membrane fusion. However, this alone is not sufficient to block phagosome-lysosome fusion (Wong *et al.*, 2011). It has been reported that *mptpA* mutant of *M. tuberculosis* was impaired for survival/growth in THP-1 macrophages and phagosomes harboring the mutant strain exhibited increased phagosome-lysosome fusion. Although unanswered questions remain in the proposed model, MptpA is clearly a key protein involved in *M. tuberculosis* inhibition of phagosome acidification and maturation and ultimately the pathogenesis of *M. tuberculosis*.

3. **Mycobacterial protein tyrosine phosphatase B (mptpB, Rv0153c)**

It has been postulated that MptpB might be secreted by *M. tuberculosis* into either the phagosome or host cytosol during infection, disrupting macrophage tyrosine kinase signaling pathways. MptpB was found to possess the unique property of triple specificity for phosphoinositides, phosphotyrosine, and phosphoserine/phosphothreonine as substrates (Beresford *et al.*, 2007). Based upon these results, it is reasonable to argue that MptpB might also be capable of disrupting host phosphoinositide and its associated signaling pathways. Although the target of MptpB within the host macrophage is yet to be identified, there is evidence demonstrating the essentiality of MptpB in *M. tuberculosis* pathogenesis (Singh *et al.*, 2003; Beresford *et al.*, 2009). It has been previously reported that *M. tuberculosis* devoid of MptpB activity was impaired for survival in IFN-γ activated macrophages and in guinea pigs (Singh *et al.*, 2003). The importance of MptpB to *M. tuberculosis* intracellular survival was also suggested by two other studies in which specific inhibitors against MptpB were shown to inhibit mycobacterial survival within murine macrophages as compared to untreated macrophages (Beresford *et al.*, 2009) (Zhou *et al.*, 2010). Nonetheless, Zhou *et al.* showed that
expression of MptpB in activated murine macrophages resulted in the reduced phosphorylation of the extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 mitogen-activated protein kinase (MAPK), which led to the decreased production of IL-6 (Zhou et al., 2010). Furthermore, MptpB expression resulted in increased Akt phosphorylation and decreased caspase-3 activation associated with apoptosis inhibition (Figure 8). Although ERK1/2 and p38 are not direct substrates of MptpB, these observations suggest that MptpB disrupts host signal transductions resulting in subversion of host immune response. Thus, it is possible that MptpB plays a key role as an immune response damper, balancing host immune detection with evasion during \textit{M.tuberculosis} infection.

\textbf{Development of post-exposure vaccination strategy}

The standard TB chemotherapy requires people to take four drugs (isoniazid, rifampicin, pyrazinamide and ethambutol) for two months followed by two drugs (isoniazid and rifampicin) for four months, and generally it is effective. Unfortunately, ‘generally effective’ does not mean ‘always effective’. Although the reasons for treatment failure vary and are always tragic, the spread of drug-resistant \textit{M. tuberculosis} is especially alarming due to non-compliance to such a prolonged regimen. Strains categorized as multidrug resistant (MDR) are resistant to at least the two first-line TB drugs (isoniazid and rifampicin), whereas those described as extensively drug resistant (XDR) are additionally resistant to fluoroquinolones and one or more of three injectable second-line drugs (capreomycin, kanamycin and amikacin). Besides, there have been recent reports of totally drug resistant TB which is very difficult and expensive to treat. Totally drug resistant TB (usually abbreviated to XXDR TB), which is resistant to all the first and second line TB drugs. Treating TB cases is clearly insufficient to interrupt disease transmission in highly endemic populations (Ottenhoff and Kaufmann, 2012). In the last four decades, Sirturo (bedaquiline) is the only TB drug approved by the FDA as part of combination therapy for adults with MDR TB when other alternatives are not possible. Hence, better preventive measures that block \textit{M.tuberculosis} transmission need to be developed, including vaccines that prevent establishment of \textit{M.tuberculosis} infection in the susceptible human host or alternatively, therapeutic vaccines that prevent progression of established infection towards active TB disease or reduce the duration of TB chemotherapy.

For the evaluation of therapeutic vaccines or post exposure vaccines, three murine models of latent \textit{M.tuberculosis} infection have been most widely used. One of the models is “low dose aerosol model” in which, mice are infected aerogenically with 5-10 \textit{M.tuberculosis} bacilli.
(Orme, 1988). Approximately 3 months later, the bacillary burden reaches 10^4 CFU/lung and remains at these levels for 15 to 18 months, after which the infection takes off and the mice succumb to TB. This model has the advantage of keeping the bacterial burden under control for an extended period of time, but it has the disadvantage of a high bacillary burden that is unlikely to reproduce the actual low rate of bacterial multiplication that is found in human latent TB. The second model, called the Cornell model, was first described in the late 1950s (McCune and Tompsett, 1956; McCune et al., 1966a; McCune et al., 1966b). In this model, mice are inoculated intravenously with 2 X 10^6 *M. tuberculosis* bacilli, and the resulting infection is treated for 12 weeks with the anti-mycobacterial drugs isoniazid and pyrazinamide beginning within 20 min after infection. Subsequently, no bacilli can be cultured from the animals’ organs for many months. However, administration of cortisone (at immunosuppressive doses) 2 to 3 months after the interruption of the antibiotic therapy causes this condition to revert, and *M. tuberculosis* can be cultured from the lungs and the spleens of 50% of the animals. Even though Cornell model has the advantage of achieving and maintaining very low numbers of the tubercle bacilli within the tissues of infected mice for many weeks, this model has three major limitations: (i) it is difficult to standardize the conditions for generating dormancy as the optimal antibiotic concentration and the treatment duration may vary from experiment to experiment; (ii) only 50% of the animals successfully treated with antibiotic develop dormant infection, which imposes a major complication in the interpretation of the results of the experiments; and (iii) most variants of the Cornell model use high doses of immunosuppressive reagents to achieve reactivation, which by definition constitutes a complication for studies designed to evaluate the host immune response during the reactivation of the disease. Thirdly, another unique model of dormant infection is established where mice are inoculated with a unique streptomycin-auxotrophic mutant of *M. tuberculosis* which recapitulates dormant infection (Kashino et al., 2006). The mutant grows unimpaired in the presence of streptomycin, however, it no longer grows but remains viable for long periods of time after substrate removal. Mice challenged with the mutant and inoculated with streptomycin for 3 weeks developed a limited infection characterized by a low bacteriological burden and the presence of typical granulomas. After substrate withdrawal, the infection was hindered but few microorganisms remained viable (dormant) in the animals’ tissues for at least 6 months. However, this model does not mimic the true picture of latency as in the case of human TB.

In this study, we have developed a mice model of latency based on the modifications of Cornell model and evaluated the immunotherapeutic potential of adjunctive immunotherapy with DNAacr (DNA vaccine encoding α-crystallin) and DNAsod (DNA vaccine encoding
superoxide dismutase) to shorten the duration of chemotherapy and to prevent the reactivation of latent infection.

For this, α-crystallin and superoxide dismutaseA were selected as antigens. Their importance in the context of TB vaccine development is described below.

1. α-crystallin (acr, Rv2031c)

α-crystallin is a heat shock protein coded by the gene hspx that is induced by anoxic stress. It is regulated by the two component regulatory system DevR (Rv3133c)/DevS (Rv3132c), in response to a hypoxic signal. It has a proposed role in the maintenance of long-term viability during latent, asymptomatic infections and in replication during initial infection. (Vordermeier et al., 1993; Friscia et al., 1995). The potential success of latency antigens was recently reported by Peter Andersen and colleagues, who showed that vaccination with a fusion protein, known as Hybrid 56 linking the Hybrid 1 (Ag85B-ESAT6) backbone to M. tuberculosis starvation/latency antigen Rv2660c induced superior protection against TB when compared to BCG or Hybrid 1 vaccination (Lin et al., 2012). In addition, α-crystallin has a crucial role in the ability of M. tuberculosis to survive in a state of non-replicating persistence within the hostile intracellular environment of the host (Chang et al., 1996; Yuan et al., 1996; Cunningham and Spreadbury, 1998). When subjected to anaerobic stress, M. tuberculosis induces a massive up-regulation of the production of this protein, which is associated with a thickened cell envelope. The antigen exists in vitro as a trimer of trimmers with a molecular weight of 149,000 ± 8000 kDa (Chang et al., 1996).

Although classified as a member of a widely distributed class of heat-shock proteins, the 16 kDa antigen has not been detected outside the M. tuberculosis complex (Verbon et al., 1992; Yuan et al., 1996). Moreover, there are several permissively recognized B cell and T cell epitopes in α-crystallin which generate an efficient immune response (Verbon et al., 1992; Wilkinson et al., 1998; Agrewala and Wilkinson, 1999). Presence of antibodies against α-crystallin in about 85% of patients provides the evidence that this protein is an immunodominant protein suggesting its role in the host defense mechanisms against M. tuberculosis (Lee et al., 1992). Besides, latently infected individuals (healthy PPD+ and household contacts) exhibit increased lympho-proliferative and IFN-γ response to α-crystallin as compared to patients with active TB (Vekemans et al., 2004). These attributes of predominant expression during mycobacterial dormancy, species specificity and immunodominance, make it a highly attractive candidate for the study of immune response, both from the point of view of protective immunity and development of novel immunodiagnostic reagents. More importantly, we have demonstrated that that DNA vaccine encoding α-crystallin
conferred a superior protection than BCG vaccination when employed either in ‘rBCG prime – DNA boost’ or ‘BCG prime – DNA boost’ regimen (Dey et al., 2011b) (Dey et al., 2011a).

2. Superoxide dismutase A (sodA, Rv3846)

Superoxide dismutase is a part of the defense mechanism, which protects pathogens from the toxic effects of reactive oxygen intermediates (Hassett and Cohen, 1989). It detoxifies superoxide anion and is capable of neutralizing oxygen metabolites released from immune cells. *M. tuberculosis* produces two SOD proteins, (i) an iron-cofactored enzyme encoded by *sodA* (*Rv3846*) and (ii) a copper-zinc cofactored enzyme encoded by *sodC* (*Rv0432*) (Zhang et al., 1991; Harth and Horwitz, 1999; Dussurget et al., 2001; Piddington et al., 2001). The production and extracellular secretion of SodA in copious amount especially by the pathogenic mycobacterial species reflects towards its involvement in the virulence of these organisms (Harth and Horwitz, 1999). Conversely, SodC is produced by most of the mycobacterial species including fast growing saprophytes as well as slow growing pathogens but at much lower levels. The relative importance of SodA over SodC is further reinforced by the demonstration that, wherein the absence of SodC did not affect the *in vivo* growth of *M. tuberculosis*, reduction in SodA production by antisense RNA resulted in a marked attenuation of *M. tuberculosis* growth and virulence (Edwards et al., 2001; Piddington et al., 2001).

Besides making an important contribution to the intracellular survival of the pathogen, it also induces an immune response in the host due to the presence of several immunodominant B-cell epitopes. Several studies have reported that many of the SodA epitopes are recognized in PPD positive individuals as well as in TB and leprosy patients (Deshpande et al., 1993; Bisht et al., 1996; Dong et al., 2004). It has also been shown to induce strong DTH responses in guinea pigs thereby suggesting the elicitation of T cell mediated responses by the host. Moreover, the linear peptide, p160-168 of *M. tuberculosis* sod A has been reported as the most immunodominant T cell epitope that is largely implicated against HLA-A2 bearing cells generating CD8+ responses (Dong et al., 2004; Zhu et al., 2011). SodA appears to be crucial for the survival of mycobacteria and may be the targets of strong cell-mediated host immune responses. In addition, previously we have reported that sod A based DNA vaccination elicited an efficient immune responses and imparted significant protection in guinea pigs (Khera et al., 2005).
REFERENCES


mouse strains: CD4 and CD8 T-cell epitope mapping and role of gamma interferon. Infect Immun 75, 4105-4115.


149. Saleh, M.T., Belisle, J.T., 2000. Secretion of an acid phosphatase (SapM) by Mycobacterium tuberculosis that is similar to eukaryotic acid phosphatases. J Bacteriol 182, 6850-6853.


in the Gambian population: implications for vaccines and immunodiagnostic test design. Infect Immun 72, 381-388.


