Critical research concepts in tuberculosis vaccine development

G. Delogu¹, R. Manganelli² and M. J. Brennan³

¹) Istituto di Microbiologia, Università Cattolica del Sacro Cuore, Rome, ²) Department of Molecular Medicine, University of Padua, Padua, Italy and ³) Aeras, Rockville, MD, USA

Abstract

A new and improved vaccine against tuberculosis (TB) would provide a powerful tool to conquer one of the most insidious infectious diseases of mankind. Protection afforded by bacillus Calmette-Guérin (BCG) has been shown to be limited and inconsistent, especially in adults that are known to transmit TB disease. In the last two decades, several new vaccines have been developed and tested with the aim to elicit robust and long-lived T-cell responses against Mycobacterium tuberculosis antigens. Although much progress has been made in the TB vaccine field, there is an urgent need to address critical research questions about TB immunity with a special focus on designing vaccines aimed at preventing infection and transmission of TB. Here, we discuss the rationale behind the current immunization strategies being implemented for TB vaccines and provide some suggestions for hypothesis driven research to encourage the development of novel TB vaccines.

Keywords: Bacillus Calmette-Guérin, disease transmission, Mycobacterium tuberculosis, tuberculosis, vaccines

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The Critical Need for an Effective Vaccine for Tuberculosis

Once considered a disease of the past, tuberculosis (TB) has re-emerged in the last 30 years as a major threat in many parts of the world. The latest WHO report estimates 1.3 million deaths in 2012 and an incidence of 8.6 million TB disease cases [1]. Globalization is changing the epidemiology of TB, with the emergence of drug-resistant strains seen as a major concern [2,3]. New tools are urgently needed to control TB at a global level and the availability of an effective vaccine will contribute to reduce TB incidence and mortality [4].

Bacillus Calmette–Guérin (BCG) remains the only vaccine available for TB [5]. It is still routinely administered in countries where TB is endemic to newborns immediately after birth because of its efficacy in preventing the most severe forms of TB in early childhood [6]. There is consensus that BCG is unable to provide significant protection against pulmonary TB in adults [7,8], which is the only form that promotes Mycobacterium tuberculosis transmission [9]. In the last decade, a renaissance in TB research has resulted in the development of many new experimental vaccines, some of which were shown to induce in animal models a protective immune response superior to that induced by BCG (Table 1) [10–13]. More than fifteen of these new vaccines have entered or completed clinical trials.

A T-cell-based Approach to TB Vaccine Design

The fundamental rationale for the development of TB vaccines designed to elicit T-cell-based immunity is based on the assumption that eliciting a strong T helper type 1 immune response specifically directed against M. tuberculosis antigens provides a rapid mobilization of T cells at the site of primary infection that can control bacterial replication and prevent progression to active disease (Fig. 1) [10]. The central role of T-cell immunity in controlling mycobacterial infection is well established as illustrated by the enhanced susceptibility to TB
TABLE 1. New tuberculosis vaccine candidates under development

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Name</th>
<th>Description</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live attenuated Mtb</td>
<td>MTBVAC</td>
<td>Unmarked double mutant missing the global regulator phoP and the phthiocerol dimycocerosates (DMCs)-biosynthetic gene famD2; enhanced protection in animal models</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>Mtb ΔsigE</td>
<td>Mutant missing the gene for the alternative sigma factor SigE; differential modulation of innate immune responses; enhanced protection in mice</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>Mtb ΔsecA2</td>
<td>Mutant missing secA2, encoding for a component of a virulence-associated secretion system, results in a proapoptotic attenuated strain that warrants enhanced CD8 T-cell priming</td>
<td>[16]</td>
</tr>
<tr>
<td>Recombinant BCG</td>
<td>rBCG30</td>
<td>rBCG overexpressing Ag85B; enhanced immunogenicity and protection in mice and guinea pigs</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>BCG:RD1-2F9</td>
<td>rBCG complemented with the ESX-1 region from Mtb; enhanced protection in animals</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>VPM 1002</td>
<td>rBCG ΔwurC-hly, deleted in the urease gene and expressing listeriolysin to promote phagosome lysis resulting in better antigen presentation, enhanced protection in mice</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>AERAS-422</td>
<td>rBCG expressing perfringolysin and several Mtb antigens, leads to phagosome lysis resulting in better antigen presentation, enhanced protection in mice; phase I terminated due to side effects</td>
<td>[20]</td>
</tr>
<tr>
<td>Reombinant NTM</td>
<td>rBCG PE_MPT64</td>
<td>rBCG overexpressing MPT64 on its surface resulting in enhanced protection in mice</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>IFEPLUS</td>
<td>Mycobacterium smegmatis ΔsecJ complemented with Mtb easJ; stimulation of protective bacterial immunity against Mtb in mice</td>
<td>[22]</td>
</tr>
<tr>
<td>Viral vectored</td>
<td>MVA85A</td>
<td>Vaccinia virus Ankara expressing Ag85A; enhanced protection in animal models, no efficacy in phase 2a trial</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>AdAg85A</td>
<td>Replication-deficient adenovirus expressing Ag85A, enhanced protection in animal models</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>AERAS-402</td>
<td>Replication-deficient adenovirus expressing Ag85A, enhanced protection in animal models</td>
<td>[25]</td>
</tr>
<tr>
<td>Protein subunit</td>
<td>H1</td>
<td>Fusion protein Ag85B-Eas6, enhanced protection in animal models; safe and immunogenic in humans</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>H56</td>
<td>Fusion protein Ag85B-EASAT-6-Rv2660c, enhanced protection in animal models, prevents tuberculosis reactivation in monkeys</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>H4</td>
<td>Fusion protein Ag85B-TB104; strong protection in animal models</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>Mtb72F</td>
<td>Fusion protein PepA-PPE18, long-term protection in non-human primates</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>ID93</td>
<td>Fusion protein Rv2608-Rv3619-Rv3620-Rv1813; increased protection in BCG-vaccinated guinea pigs after boosting</td>
<td>[30]</td>
</tr>
<tr>
<td>Therapeutic vaccine</td>
<td>Mycobacterium vaccae</td>
<td>Killed M. vaccae; improves the efficacy of chemotherapy</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>RUTI</td>
<td>Liposomes containing detoxified fragmented Mtb cells; reduces treatment time in animal models</td>
<td>[32]</td>
</tr>
</tbody>
</table>

*Only one relevant for each experimental vaccines has been selected. Abbreviations: BCG, bacillus Calmette-Guérin; Mtb, Mycobacterium tuberculosis; NTM, nontuberculous mycobacteria.

FIG. 1. Schematic showing a model of a typical anti-mycobacterial immune response based on T cells that may be induced by bacillus Calmette-Guérin and some of the vaccines currently under development. These vaccines elicit a T-cell immune response directed against Mycobacterium tuberculosis (Mtb) antigens and rapid mobilization of these T cells and recruitment of other leucocytes at the site of bacterial replication warrants a robust and more effective control of Mtb compared with non-immunized subjects. IFN-γ, interferon-γ; PMN, polymorphonuclear cells.

infection and disease of subjects with impaired T-cell responses, such as those with primary or acquired immunodeficiencies as observed in HIV-infected subjects or patients undergoing anti-tumour necrosis factor therapy [34,35]. Many studies in mice have clearly demonstrated the essential role of T cells in controlling M. tuberculosis growth in vivo and the pivotal role of interferon-γ, a cytokine that is integral to the cell-mediated immune response [36–38]. Additionally, it is well established that T-cell responses are also involved with the immunopathology and tissue damage associated with TB disease [39,40].

An example of a T-cell-based TB vaccine strategy is the development of the modified vaccinia Ankara (MVA) strain expressing the mycobacterial antigen Ag85A. This vaccine candidate, called MVA85A, boosts anti-tuberculocytic activity in animals previously immunized with BCG that is associated with induction of Ag85A-specific, interferon-γ-secreting T cells [41–43]. These animal models were specifically designed to test immunization strategies that would be considered feasible in human prime-boost studies. Subsequently, MVA85A was found to be safe and immunogenic in children [44,45] and a double-blind, randomized, placebo-controlled, phase 2b trial
was performed in South Africa, a country with a high burden of TB, to measure the activity of MVA85A in previously BCG-immunized infants [23]. Boosting with MVA85A was well tolerated and an Ag85-specific CD4-positive T-cell response, predominantly expressing interferon-γ, tumour necrosis factor-α and interleukin-2, was measured in ex vivo assays in children boosted with MVA85A but not in the placebo group, yet no efficacy against TB or M. tuberculosis infection was measured in the MVA85A immunized children compared with the placebo group [23].

The lack of any measurable improved protective activity in children for MVA85A, one of the most promising vaccines against TB, compared with BCG, has been a wakeup call for the TB vaccine community. Since many of the new TB vaccines in clinical trials or in advanced preclinical testing are using a similar immunological rationale, geared to eliciting a strong T-cell response directed against M. tuberculosis antigens [46], in-depth study of the immunological responses and efficacy of other vaccines designed to enhance T-cell-based immunity will be critical in assessing the true utility of this strategy.

Genome comparisons of strains representative of the global diversity of the M. tuberculosis complex have demonstrated that known human T-cell epitopes in M. tuberculosis are more conserved than essential genes [47]. These findings suggest that the immune responses elicited by these T-cell epitopes, while essential for the survival of the individual human host, might also be beneficial for the bacteria [47,48]. T-cell responses may contribute to human-to-human transmission of M. tuberculosis, perhaps by directly causing the lung tissue destruction and cavitation that facilitates spread of the bacilli [47,49]. The implications of these findings for the development of new vaccines against TB are particularly relevant, because this theory suggests that vaccines aimed at inducing strong T-cell responses against immune-dominant M. tuberculosis antigens may paradoxically increase transmission. The lack of valid animal models of disease and transmission mimicking human TB is a major hurdle to dissecting the differential role of T-cell immune responses and identifying key components associated with protection or disease. This is particularly true considering that most, if not all, of the TB vaccines tested to date are aimed at preventing disease rather than infection.

None of the vaccines evaluated so far has been able to reproducibly induce sterilizing immunity and prevent M. tuberculosis infection in animals, which inevitably leads to chronic/persistent infection or overt disease. The lack of an immunological correlate of protection makes it necessary to evaluate several parameters that measure vaccine activity in animal models. Protection is measured as a reduction in bacterial loads in lung and spleen tissues and quantitative/qualitative lessening of the lung tissue damage as assessed by histopathological analysis in vaccinated compared with naive animals, together with BCG-immunized groups used as the reference standard [50]. Overall, it is unclear whether and how any of the responses to vaccines measured in animals translate into a confident assessment of vaccine protection against TB infection or disease. It also remains unclear whether improvements in the degree of reductions in mycobacterial loads would predict enhanced ability to prevent disease development or infection in humans. The results of Tameris et al. [23] in part challenge the notion that a vaccine capable of producing enhanced protection in preclinical animal models will be more efficacious in humans, suggesting that the present parameters for selection of TB vaccine candidates might be inadequate.

**Targeting M. tuberculosis Transmission**

A major limitation of BCG vaccine is its inability to provide any measurable effect on limiting transmission of the M. tuberculosis organism. This present the danger that new TB vaccine approaches based on BCG or on BCG prime-boost strategies may not interrupt transmission, thereby failing to provide the public health benefits that are classically associated with vaccination [51]. The outcomes of transmission of M. tuberculosis are a function of the active pulmonary TB patient’s infectiousness and of the newly infected host’s susceptibility (Fig. 2a). Progression to disease, also depends upon bacterial replication, tissue damage and cavitation in the lung tissue, which is the result of a complex and dynamic host-pathogen interplay and which potentially could be influenced by a vaccine-induced immune response [52]. A vaccine capable of limiting extensive tissue damage, preventing cavitation in the lung, or diverting bacteria towards extrapulmonary sites where they can be successfully scavenged may limit transmission [51]. Unfortunately, the relevant information and comprehensive understanding of the immunological determinants associated with tissue destruction and lung cavitation required to design a vaccine specifically aimed at blocking transmission are lacking [53]. The identification of antigens expressed by M. tuberculosis in the lung tissue during chronic infection [54], the characterization of their immunomodulatory role and their use in vaccines could limit cavitation and bacterial release through the airways. Candidate antigens for this approach may exist and could include the PE_PGRS proteins, which are unique to the MTB complex and a few other pathogenic mycobacteria, have powerful immunomodulatory properties and are over-represented in granulomas of M. tuberculosis-infected guinea pigs [54–56].

The initial steps by which exposure to M. tuberculosis results in the establishment of infection are not completely understood and have mainly been inferred from studies measuring
conversion of contacts to pulmonary TB patients assessed by the tuberculin skin test. Estimates suggest that infection occurs in only 20–50% of subjects who inhale the tubercle bacillus [57]. The introduction of the more sensitive interferon-γ release assays (IGRAs) to detect and monitor *M. tuberculosis* infection has provided a new view on the dynamic role of the T-cell responses in the early steps of infection [58,59]. It has been shown that IGRA conversions among household contacts may, in a few subjects, eventually revert to negative and this event is more likely to occur in individuals who are tuberculin skin test negative and who show levels of interferon-γ responses in the Quantiferon assay slightly above the recommended threshold [60]. This dynamic T-cell response has led to the concept of ‘transient’ infection, defined as an acute resolving infection with the complete clearance of the bacilli, an outcome inversely correlated with the dose of infection [61]. These findings provide experimental evidence suggesting that *M. tuberculosis* is relatively difficult to transmit and that a vaccination strategy that specifically targets the transmission process, either by reducing bacterial spreading and/or enhancing antibacterial activities in the early steps of infection, may have a significant impact on the control of TB. To date, the lack of reliable animal models has prevented the evaluation of vaccine strategies aimed at blocking TB transmission.

**What about Antibodies?**

Most approved vaccines against respiratory pathogens are believed to mediate protection by generating an antibody response that neutralizes the infecting inoculum on mucosal surfaces, so reducing the pathogen’s transmission [62]. The role of antibodies in TB pathogenesis has been questioned for more than a century and the common assumption is that humoral responses play little or no role in protection against TB or intracellular pathogens in general [63,64]. This concept is supported by the observation, in animals and humans, that antibody titres against mycobacterial antigens are highest during TB disease and correlate with *M. tuberculosis* burden, not with protection against infection [65]. The role of antibody-mediated immunity against TB has been extensively reviewed [64] and, based on an assessment of the experimental evidence, two potential mechanisms of protection have been proposed by the authors. Antibodies could exert their activity by interacting with host immune mediators or by binding mycobacterial antigens with immunomodulatory properties and promoting their clearance, thereby modulating anti-inflammatory or pro-inflammatory responses in infected hosts, which may impact disease outcome [64,66]. In the
context of the damage-response framework [67], antibodies may help to limit tissue damage and cavitation or may promote *M. tuberculosis* dissemination and TB worsening [64]. More extensive studies of the humoral response during *M. tuberculosis* infection may lead to new strategies to limit tissue destruction in the lung tissue and reduce TB transmission.

Antibodies may also exert their protective activity by directly binding bacterial surface components. Phagocytosis of opsonized *M. tuberculosis* has been shown to enhance intracellular killing in macrophages [68,69], which may increase bacterial clearance following infection. Antibodies directed against HBHA, a mycobacterial adhesin, were shown to reduce bacterial dissemination from the site of primary infection in mice [70]. A monoclonal antibody against arabinomannan increased survival of *M. tuberculosis*-challenged mice [71] and antibodies directed against the non-proteinaceous surface component lipoarabinomannan were shown to exert a protective activity [72]. Although studies such as these have suggested that antibodies may have a role in a protective response against TB, the results of passive immunization studies using purified antibodies have been equivocal [64]. Nevertheless, more attention should be given to the development of vaccines specifically aimed at inducing humoral responses as well as investigating the role of B cells in TB immunity.

**Critical Research Questions**

Since infection with *M. tuberculosis* occurs with inhalation of a few bacilli, antibodies that attack the pathogen and/or enhance antimicrobial defences during these early steps of infection have the potential to elicit anti-infective immunity. To implement and test this hypothesis there are several questions and areas of research that need to be addressed (Fig. 2b). These include the following:

1. A better characterization of the *M. tuberculosis* surface constituents, with emphasis on proteins involved in the early steps of infection, is necessary to identify candidate antigens for the development of anti-infective vaccines against TB. For instance, it is well known that entrance of *M. tuberculosis* into macrophages through the CR3 receptor enhances the chance of mycobacterial survival in the intracellular niche [73]. Identification of the bacterial determinants involved in this receptor-mediated entry may open new avenues to develop antibody-based vaccine strategies. Analysis of surface glycolipids and polysaccharides, which have been neglected as vaccine antigens, is also needed.

2. Currently, in vitro and in vivo experiments aimed at testing new vaccines are carried out with *M. tuberculosis* grown in liquid culture, in media containing detergent to limit bacterial clumping. These conditions alter the natural organization of the mycobacterial surface. Ideally, experiments used to test antibody-based strategies should use bacteria with a well-preserved surface, resembling that of bacilli released by a patient with active pulmonary TB. Bacteria released by superinfected and dying macrophages or obtained by homogenization of lung tissues from infected animals may offer a better model to measure the ability of antibodies to bind surface constituents.

3. The animal models currently used to assess vaccine efficacy rely on a low-dose aerogenic infection with *M. tuberculosis* (c.100 CFU/animal). Protection is measured as a reduction in Log CFU/organ of *M. tuberculosis* in vaccinated animals compared with control animals. The protective activity of anti-infective vaccines ideally should be measured in models that better mimic human transmission, such as challenge models using a very low dose of infection (<10 CFU/animal) preferentially administered in multiple, consecutive doses. In these models, protection should be measured as the per cent of infected animals in the vaccinated animals versus control animals rather than as reduction in CFUs. This approach is similar to those routinely performed when testing anti-infective vaccines against other bacterial pathogens [74,75]. In this experimental model, greater numbers of animals need to be infected with *M. tuberculosis* and infection could be identified by a simple IGRA-like assay.

4. The identification of important *M. tuberculosis* antibody-producing surface constituents involved in the interaction with host components during the early steps of infection should be better defined in human populations. In this context, it would be important to investigate whether uninfected close contacts of TB patients, or subjects in whom a transient infection has been demonstrated, have antibodies against certain antigens of *M. tuberculosis* in the sera or mucosal surfaces (bronchiolalveolar lavage).

Although progress has been made in the TB vaccine field over the past decade and a first generation of vaccines is being tested in clinical trials, an urgent need to address critical research questions about TB immunity still exists. One primary question needs to be addressed: what responses to a vaccine need to be induced to control the infection and transmission of TB? Progress on the next generation of TB vaccines will probably require creative but testable responses to this question.

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Transparency Declaration

The authors have no conflicts of interest to declare.

References

26. van Dissel JT, Soosawala D, Joosten SA et al. Ag85B-ESAT-6 adjuvanted with IC31(R) promotes strong and long-lived Mycobacterium tuberculosis specific T cell responses in volunteers with previous BCG vaccination or TB infection. Vaccine 2011; 29: 2100–2109.


