CHAPTER 5.2

TB VACCINE DEVELOPMENT: A BRIEF BACKGROUND

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The achievement of an effective vaccine is based on the concerted effort of many participating persons and institutions, from fundamental research up to the final product.

‘Sea, purest of water to the fish, will never satisfy the human thirst.’

The Resting Place of Fire
(Gift of Heraclitus)
Jose Emilio Pacheco

The Great Car Race
Ángel Acosta León
Oil on masonite; 244.5 × 122 cm
Collection of the National Museum of Fine Arts, La Habana, Cuba

1Uli Fruth is a staff member of WHO. The author alone is responsible for the views expressed in this publication, and they do not necessarily represent the decisions, policy or views of WHO
INTRODUCTION

Every year, about 1 million HIV negative individuals die as a consequence of pulmonary TB and many hundreds of thousands more succumb to TB as a direct consequence of the breakdown of immunity caused by HIV (1). These deaths occur despite the availability of efficient drugs and a vaccine, BCG, which is the most widely used of all childhood vaccines. The effectiveness of the BCG vaccine can be described at best as variable and drug treatment is long and burdensome, optimally requiring direct observation by a health worker. This and the fact that (a) diagnosis of infection with MTB, at least in developing countries, is often made late when bacteria already appear in the sputum and are being spread to previously uninfected contacts; (b) reinfection or reactivation of MTB occurs in drug-cured TB patients; and, (c) MDR-MTB strains commonly result from improper adherence to chemotherapy regimens, justifies the need for an effective vaccine to help control the global epidemic of TB.

The search for new, improved TB vaccines is a relatively young field. No research activities aimed at the development of a new vaccine worth mentioning are recorded between the introduction of BCG vaccine in the 1920s and the late 1980s, when TB, in the wake of the rising HIV/AIDS pandemic, made a frightening comeback. Nevertheless, numerous promising new approaches have been developed during the last two decades. Advances in gene and antigen identification, availability of genome sequences, a greater understanding of immune mechanisms possibly able to control mycobacterial disease, the development of adjuvants and delivery systems to stimulate T cell immunity, and increased funding from the public as well as the private sectors are some of the reasons for progress in this area. The most advanced of the new TB vaccine candidates are now entering clinical evaluation. This chapter will try to give an overview over this very dynamic field of research and also highlight potential bottlenecks, both scientific and financial.
TB PATHOGENESIS AND HOST RESPONSE

An understanding of the pathogenic mechanisms of MTB infection and colonization of susceptible hosts and the role of the subsequent host immune response to the invading organism in the progression of TB is crucial to the development of better vaccines and treatments for this widespread disease. It is important to appreciate that the pathology of TB (particularly in the lungs), although initiated by the invading bacterium, MTB, is also a result of the complicated immune response to this intracellular organism. Our current understanding suggests that a cascade of host defense mechanisms is triggered when a relatively small number of inhaled MTB reach the terminal airspaces of the lungs and are ingested by alveolar macrophages. This initial event is followed by a phase of logarithmic growth of the bacilli at the site of infection, their spread to the proximal lymph nodes, and eventual dissemination to other sites in the body. The process of replication and dissemination is commonly controlled by the onset of an effective immune response. The typical manifestation of cellular immunity against TB is the formation of immune-dependent granulomas (or tubercles), consisting of a core of MTB-harbouring macrophages, and surrounded by a layer largely composed of lymphocytes (2). It is, however, important to understand that the bacteria walled off within the granuloma are almost never completely eliminated and infection may reactivate at a later date. The lifetime risk for immunocompetent individuals for this to happen is estimated at around 5–10 per cent, whereas the risk of reactivation for an immunocompromised, for example, HIV-infected, individual is thought to be around 10 per cent annually (3).

It is widely accepted that protective immunity against TB relies on the activation of T cells rather than B cells (4). Within the T cell ‘family’, it is the CD4+ T cells which are thought to be the key in fending off TB. However, other T cell types such as CD8+ and γδ T cells are known to participate in the antimycobacterial immune response, but their relative importance during the progressive stages of the disease remains elusive. T cells are known to exert their function, at least partly, through secretion of a number of cytokines (5, 6). In particular IFN-γ and IL-12 have been ascribed beneficial roles in protection against TB and exceptional susceptibility to TB has been described in human individuals who are genetically deficient for IFN-γ receptor, the IL-12 receptor or IL-12 (7).
FUNCTIONAL CHARACTERISTICS OF NEW TB VACCINES

What is needed is probably not one but more likely two or even three new TB vaccines with different profiles: a priming vaccine to replace BCG to be given early in life and before exposure to MTB, another one to boost antimycobacterial immune responses either early or later in life when latent TB is potentially installed, and possibly a therapeutic vaccine against active TB (8). It may be possible that a vaccine can be identified which covers several of these functional profiles, but this will not be automatically the case for all vaccine candidates. Thus, it is known that live BCG does not boost anti-TB immunity in latently infected or previously BCG-immunized human individuals or animals.

**Priming TB vaccines**

This type of TB vaccine, sometimes referred to as ‘pre-exposure’ TB vaccines, of which BCG is the prime example, is intended for use in newborns or young infants, that is, at a time point when the individual’s immune system has not yet been exposed to natural infection with MTB or other mycobacteria. All TB vaccine candidates which are currently in preclinical or early clinical development were selected in protection studies in unexposed animal models, that is, mimicking neonatal vaccination. Current thinking implies that live TB vaccines such as ‘old’ BCG, improved BCG, or rationally attenuated MTB would be used as ‘first contact’ priming vaccines. However, there are also proponents for an inverted sequence, that is, non-living vaccine at birth followed by BCG or another live vaccine at 3–4 months of age (9). Such an approach aims to improve the immunogenicity of BCG, but more importantly, also to avoid the severe adverse events observed in some HIV-infected infants who had been vaccinated with BCG at birth — a time point at which HIV infection cannot be diagnosed (10).

**Booster TB vaccines**

Booster TB vaccines’, sometimes referred to as ‘post-exposure TB vaccines’, are vaccines that can be given together with other childhood vaccines during the first year of life (‘early boost’) and also, at almost any time point, to schoolchildren, adolescents, or adults, when the individual has either been vaccinated, for example, with ‘old’ or ‘improved’ BCG, or latently infected with MTB (or other mycobacteria) or both (‘late boost’). TB vaccines to be used against firmly established latent TB may require a different set of antigens than the ones that
are expected to be active against primary infection and the type of immune response induced may differ. Also, the fact that later in life TB can arise both from endogenous reactivation and from exogenous reinfection — with the latter representing up to 75 per cent of all TB relapse cases in high burden countries (11) — may need to be reflected in the antigenic composition of a potent post-exposure TB vaccine. The difficulty in reliably reproducing latency in animal models is an obstacle to the development of post-exposure TB vaccines as are safety concerns over potential immune exacerbation induced by vaccination of latently infected individuals, sometimes referred to as ‘Koch’s reaction’ (12). It must be noted that improved protection against TB through the action of a booster TB vaccine remains, at present, a hypothetical concept, for which no proof of principle exists. This is in contrast with priming TB vaccines, where the fact that neonatal BCG is protective in some populations or age groups is taken as an indication that it should be possible to improve on whatever protection BCG affords. While indeed at least one of the new booster TB vaccines has been shown to boost pre-existing anti-mycobacterial T cell immune responses (13), that is, in a post-exposure situation, it is currently unknown if these booster responses will actually translate into improved protection against TB-induced pathologies. TB vaccine that are effective in a situation where TB infection is established may be the more difficult ones to develop, but they are also the ones that are most urgently needed in order to deal with those estimated two billion individuals who are thought to be already infected with MTB and where an effective ‘post-exposure’ vaccine could make a huge impact in a short period of time. Obviously, a vaccine that could be used in mass vaccination campaigns would be particularly desirable in this context.

Therapeutic TB vaccines

Therapeutic vaccines, that is, those that are to be given to individuals with active TB disease, represent a special case of the above-mentioned post-exposure vaccines. The general idea is not to use these vaccines as stand-alone agents, but rather as adjunct to antibiotic treatment, with the aim of shortening the duration of anti-TB chemotherapy. Inactivated mycobacteria as well as a DNA subunit vaccine encoding for a mycobacterial heat shock protein are being proposed for this purpose (14, 15).
NOVEL TB VACCINE CANDIDATES

Genetically modified mycobacteria

An argument can be made that since BCG is widely used, has a good safety record, and provides some protection against non-pulmonary forms of TB in infants, we should develop a better BCG. Three main approaches to genetically improve BCG are currently underway: (a) overexpression of highly immunogenic antigens (16); (b) reconstitution of genetic segments (‘RD1’) which are lost during the attenuation of M. bovis to create BCG (17); and (c) introduction of secreted pore-forming proteins from other bacteria species such as Listeria monocytogenes, which facilitate an intracellular process called ‘endosome-escape’ and are known to induce a broader type of immune response, including in particular higher CD8+ T cell frequencies (18). Attempts to combine some of the above approaches are ongoing, for example, antigen overexpression plus ‘endosome-escape’ (19). Two recombinant BCGs have been tested in humans so far: VPM 1002 and Aeras 422. Both are expressing pore-forming proteins, in the case of VPM 1002 listeriolysin, for Aeras 422 perfringolysin (from Clostridium perfringens). The latter also overexpresses several proteins of MTB. While VPM has advanced into phase II trials in South Africa, the development of Aeras 422 is currently on hold, due to the unexplained occurrence of shingles infections in two young adults during a phase I trial of that vaccine (personal communication D. Hoft). To this day, it is unknown which antigenic shortcomings render conventional BCG suboptimal as a vaccine. The fact that BCG’s ‘parent’ organism, M. bovis, has primarily evolved in an adaptation to bovine rather than human hosts is cited as one possible reason. This assumption has sparked numerous efforts to attenuate the actual human pathogen, MTB. All approaches to develop rationally attenuated live MTB vaccines that are decreased in virulence but persistent enough to elicit effective immune responses are still in an early phase of vaccine development. Examples include candidates containing regulatory deletion mutations in the phoP locus (20), in the RD1 virulence locus, as well as metabolic mutations such as pantothenate auxotrophy (21). An improved BCG or attenuated MTB has an advantage in that it may be accepted more readily into the existing global BCG immunization programme than the other new vaccines. It is indeed planned that such live mycobacteria would replace ‘old’ BCG as the neonatal ‘priming’ vaccine in the immunization schedules to be designed for the different combinations of new products.

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2A complete overview of all TB vaccines known to be in the pipeline can be found under: http://www.stoptb.org/wg/new_vaccines/documents.asp
Adjuvanted subunit vaccines

Protein subunit vaccines have shown to be powerful vaccines against other diseases, for example, hepatitis B or human papillomavirus and, due to their ease of standardization, and safety in the immunocompromised host, are certainly the first choice of the vaccine industry. In this context, proteins secreted by MTB have received special attention as subunit vaccines (but also as ‘add-ons’ in new live vaccines) because such antigens are among the first molecules of the pathogen to be encountered by the human immune system after infection (22). There are several adjuvanted protein subunit vaccines currently in clinical trials: M72 is a recombinant fusion construct composed of the MTB 39a PPE protein and a 32kDA protease (23). The M72 vaccine in AS01A adjuvant is currently in Phase II clinical trials, as is the second example, a TB subunit vaccine designated Hybrid-1 based on a fusion protein consisting of two immunodominant antigens: ESAT-6 and Ag85B in IC-31 adjuvant. Both vaccines were selected based on good immunogenicity and protection data from animal models and have in the meantime been shown to be safe and immunogenic in early human trials (24). HyVac-4/Aeras 404, a variant of the above Hybrid-1, is currently undergoing Phase 1 clinical safety evaluation addition. Moreover, proteins that are differentially expressed by MTB under low oxygen pressure, that is, conditions similar to those that the bacteria find during the latent phase inside their human host cell, are finding their way into ‘multistage’ vaccine candidates such as hybrid 56/IC-31 and ID93/GLA-S and are now being tested as antigens to provide protection against reactivation of TB (25, 26).

Virus-vectored vaccines

Two vectored vaccine approaches are receiving current attention, both of which employ constructs that have already been used for development of HIV/AIDS and malaria vaccines. The first is an MVA (modified vaccinia Ankara) vector expressing MTB antigen 85A (M85A). In mice and humans, M85A strongly boosts BCG-induced CD4+ and CD8+ T cell responses specific for M85A. The product has completed Phase I trials in both the UK and The Gambia with promising results (13) and, with a completed Phase IIb Test-of-Concept clinical trial (follow-up completed in July 2013) in South Africa, is the new TB vaccine furthest along in clinical testing. Results of this trial are expected to become publicly available in the first quarter of 2013 (personal communication J. Shea). The second vectored vaccine approach uses an adenovirus vectors to express mycobacterial proteins, one of which, AERAS-402/ Crucell Ad35 is undergoing Phase IIb ‘Test-of-Concept’ clinical evaluation (27), while the other, Ad5-85A is in Phase I safety evaluation (28). The current plans for virus-
vectored vaccines — as well as the above-mentioned adjuvanted subunit vaccines — imply their clinical evaluation and eventual use as either ‘early’ boosters of a neonatal vaccination with live mycobacteria at one–six months of age or as ‘late’ boosters in a post-exposure situation in schoolchildren, adolescents, or adults.

Other approaches

Since the protective mechanisms against TB are not fully elucidated, numerous other largely empirical approaches are currently in the vaccine discovery pipeline. None of the concepts described below has so far been tested in human clinical trials. Lipid-containing non-protein antigens, known to be recognized by the so-called non-classical T cells, are tested in animal models (29). Conjugate vaccines against carbohydrate-containing cell-wall components of MTB are being developed to stimulate antibody production against the pathogen (30) based on a limited number of observations describing a protective role of antibodies. Finally, as mentioned above, killed mycobacteria and DNA subunit vaccines are being considered as therapeutic vaccines. The most recent products include killed MTB organisms that were grown under conditions of oxygen starvation for enrichment in latency antigens (RUTI) and an adjuvanted DNA subunit vaccine that encodes a mycobacterial heat shock protein, HSP65 (13, 14, 31).

TB VACCINE DEVELOPMENT STRATEGY

TB vaccine development is an integral element of the Global Partnership to Stop TB, a network of international organizations, countries, public and private sector donors, governmental and non-governmental organizations, and individuals that aims to accelerate social and political action to stop the spread of TB. The Stop TB Partnership has recently developed the Global Plan to Stop TB 2001–2015, which contains strategic plans of all seven Working Groups of the Stop TB Partnership, one of which is devoted to TB vaccine development (32). The main goal of the new vaccines component of the Global Plan to Stop TB 2011–2015 is to prevent all forms of TB in all age groups through the development of safe, effective and accessible vaccines that are also safe for people with HIV. It estimates that one or more new TB vaccines could become available at a 2020 time horizon. The strategic plan details seven objectives to achieve this goal.
Objective 1: Maintain a robust TB vaccine pipeline by supporting research and discovery

Basic research in the fields of immunology and molecular biology is critical for the development of new TB vaccines. Beyond more fundamental research into the pathogen and the host response which will profit all areas of TB tool development, vaccine development specifically requires increased efforts in the following areas of strategic research:

- determining the characteristics, type and differences of immune responses to TB among people who are naturally protected, those who are immune-compromised, and those who are receiving TB drugs;

- determining the immune changes that cause and/or signal recurrence of active TB in patients who have already experienced the disease;

- determining which components of MTB are typically recognized by the immune system, even though the pathogen may not be eliminated.

Objective 2: Conduct research to identify correlates of protection, and preclinical studies to assess new TB vaccine candidates

It is likely that, as current candidates move through clinical trials, the experience gained will contribute to development of new candidates in an iterative manner. In parallel, further immunological research is needed to develop standardized preclinical and nonclinical assays for new TB vaccines, and to identify correlates of protection to be used in Phase III trials.

Objective 3: Ensure availability of vaccine production capacity by expanding manufacturing facilities for TB vaccines

It takes 4–5 years to build a vaccine manufacturing facility and prepare it for commercial production. Some manufacturing capacity already exists, but investment is needed to ensure that vaccines can be manufactured to meet international regulatory standards and ensure sufficient size for production and worldwide distribution. Manufacturing of live TB vaccines such as a modified BCG or attenuated MTB presents a particular challenge since it may require dedicated facilities and staff.
Objective 4: Build capacity for large-scale clinical trials (phases II and III) of TB vaccine candidates at field sites in TB endemic countries

Large-scale vaccine trials need to be conducted in areas with a high burden of disease as incidence of disease must be sufficient to determine efficacy of a vaccine and its safety in large populations. Epidemiologic research is needed in key target groups of interest for new TB vaccines, including infants, adolescents, and people living with HIV. Multiple trial sites are necessary.

Objective 5: Conduct phases I, II and III clinical trials of TB vaccine candidates

Evaluation of vaccine candidates requires a series of clinical trials of increasing size, complexity and cost, to progressively evaluate safety, immunogenicity and, finally, efficacy. Clinical trials — and particularly large-scale Phase III efficacy trials — are the most costly component of TB vaccine research. Ensuring investments in clinical studies is a major challenge for TB vaccine research.

Objective 6: Develop delivery, regulatory and access strategies for new TB vaccines

New strategies are needed for establishing efficient regulatory pathways for new TB vaccines. Studies are also needed to understand the economic and public health impact of new TB vaccines. Vaccine marketing analyses are required to advocate for acceptability of new TB vaccines and to keep vaccines affordable.

Objective 7: Build support for TB vaccine development and uptake through advocacy, communications and resource mobilization

Global, country and community support for new TB vaccines development is essential to increase investment in TB research and to gain support in countries where clinical trials are being conducted. This support and awareness will be raised through participation in high-level forums and relevant conferences, meetings and events, stakeholder outreach, recognition of the important role of TB vaccines as
part of a comprehensive response to the TB epidemic in high-level, international, national and community-led calls to action, increased media attention, and the development of materials that are suitable for the global, national, regional and community level.

Based on the premises of the global plan, key stakeholders in TB vaccine development have subsequently developed a strategic blueprint for TB Vaccine research and development (33). The Blueprint suggests a series of actions which expands and detail the objectives of the global. It has undergone extensive stakeholder consultation, e.g. at the Second Global Forum for TB Vaccine Development (Tallinn, Estonia; 21-24 September 2010) and been presented to WHO’s Strategic Advisory Group of Experts, the organization’s advisory group on immunization policy, in November 2011.

**CHALLENGES**

**Scientific and operational challenges**

The major factor that could preclude achievement of the 2015 target relates to the scientific uncertainty about protective immunity to TB and our current lack of experience with new TB vaccines in human populations. Vaccine-induced immune or functional parameters, such as antibody threshold levels, which could be used as surrogate for a clinical end point (surrogate or correlate of protection) have not yet been defined for vaccine-mediated protection against TB. Moreover, our knowledge of the relevance of protection experiments in animals — which are used to select antigens for clinical evaluation — for humans is very limited in pre-exposure situations and completely absent for animal models of latent TB. In spite of recent advances in our understanding of host responses to MTB infection and TB disease, we may nevertheless be unable to identify amongst the current vaccine candidates ones that provide consistent protection against TB. Thus, those may prove right who argue that a vaccine’s capacity to modulate cellular immune responses, for example, suppress the Th2 pathway, may in the end be more important than the TB antigens contained in it (34). Therefore, the dual strategy of maintaining support for relevant activities in vaccine discovery research while maximizing the number of candidates introduced into clinical trials provides the optimal means of increasing our chances for developing an effective vaccine. At a more operational level, diagnosis in infants and children, who are main targets for new vaccines and therefore indispensable in clinical testing, is demanding. Definitive diagnosis may be possible in no more
than 50 per cent of suspect cases. This obstacle, together with the extreme scarcity of sites where annual TB incidence rates allow the performance of vaccine efficacy trials, will have a major influence on where an efficacy trial in that population can be organized, how many individuals will have to be enrolled and, eventually, how expensive the performance of the trial will be.

Financial uncertainties

Vaccine development is expensive. The Global Plan to Stop TB 2011–15 has identified a requirement of US$ 1.9 billion. Despite impressive commitments by the public sector and philanthropy, a funding gap remains of at least 60 per cent of the total research and development (R&D) fund required to achieve the objectives of the TB vaccine development plan in time. The three areas where resource mobilization needs are most pressing are in the areas of (a) maintenance of the vaccine discovery pipeline; (b) performance of clinical trials, in particular Phase IIb and III trials; and, (c) the creation of an enabling infrastructure. The reasons are different for each category. Maintenance of a broad basic research infrastructure is per se very expensive. Advanced clinical trials are usually financed by the pharmaceutical industry. However, as with the development of many new vaccines and drugs against diseases of poverty, commercial investment is negligible due to the estimated small size of the market for these innovative, but expensive-to-develop products. Finally and paradoxically, the elements of an enabling infrastructure are usually inexpensive and, therefore, donors oft en shy back from the administrative burden of funding these modestly sized but generally high impact activities.

CONCLUSION

The main objective of this overview chapter is to highlight the main trends in TB vaccine development. These registers of research activities are not exhaustive, but they reflect the highly complex technologies and strategies that are being attempted in the development of new vaccines against TB. The following chapters will provide more extensive analyses of some of these strategies in the hope that some of these pipeline candidate vaccines will be able to enter or complete clinical trials in the future.
REFERENCES


