One of the control measures of TB of higher potential impact
is the achievement of an efficacious vaccine

Tidal Waves – Ileana Mulet
Oil/canvas
(83 x 63 cm)

'...The artifices and naïvety of man go on
without end....'

The Golem (Poem) – Jorge Luis Borges

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Tuberculosis Vaccine Development: A Brief Background

Uli Fruth*

Introduction

Every year, about 1.7 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. 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

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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 [1]. 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 [2].

It is widely accepted that protective immunity against TB relies on the activation of T cells rather than B cells [3]. 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 (4, 5). 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 (6).

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 (7). 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 inversed sequence, that is, non-living vaccine at birth followed by BCG or another live vaccine at 3–4 months of age (8). 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 (9).

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 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. 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 (10)—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' (11). 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 antimycobacterial T cell immune responses (12), 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 (13, 14).

Novel TB Vaccine Candidates[a]

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 (15); (b) reconstitution of genetic segments ('RD1') which are lost during the attenuation of *M. bovis* to create BCG (16); and (c) introduction of secreted pore-forming proteins from other bacteria species such as *Listeria moncytogenes*, 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 (17). Attempts to combine some of the above approaches are ongoing, for example, antigen overexpression plus 'endosome-escape' (18). The only recombinant BCG that has been tested in humans so far is the rBCG30, a live vaccine that is genetically engineered to produce large amounts of a 30kDa protein (Ag85B). A Phase I study of rBCG30 in the USA showed good safety of this candidate vaccine. Another recombinant BCG of type (c) above has entered clinical evaluation in 2008. 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 (19), in the RD1 virulence locus, as well as metabolic mutations such as pantothenate auxotrophy (20). 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.

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

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[a]A complete overview of all TB vaccines known to be in the pipeline can be found under: http://www.stoptb.org/wg/new_vaccines/

stability, 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 (21). There are two 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 (22). 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 (23). While still in a rather upstream phase of development, it should be mentioned that 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 now being tested as antigens to protect against reactivation of TB. A mycobacterial protein termed α-crystalline homologue is the 'flagship' representative of that most interesting group of experimental latency or dormancy antigens (24).

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 (12) and, with an ongoing Phase IIb Test-of-Concept clinical trial (initiated in May 2009) in South Africa, is the new TB vaccine furthest along in clinical testing. The second vectored vaccine approach uses an adenovirus vector to express several mycobacterial proteins and that has entered Phase I clinical evaluation in late 2006 (25). 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 (26). Conjugate vaccines against carbohydrate-containing cell-wall components of MTB are being developed to stimulate antibody production against the pathogen (27) 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 (12, 13, 28).

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 2006–2015, which contains strategic plans of all seven Working Groups of the Stop TB Partnership, one of which is devoted to TB vaccine development (29). The overall objective of the Working Group is to have a safe, effective, licensed vaccine available at reasonable cost by 2015. The strategic plan details seven key actions to achieve this goal.

Key Action 1: Maintain and Improve BCG Vaccination Programmes

It is anticipated that BCG will remain the cornerstone of TB vaccination programmes, with the next generation of new vaccines being introduced as an addition to existing or new BCG vaccines. The better the implementing infrastructure will be set up, the easier and faster it will be to introduce new TB vaccines into an existing system rather than having to create one from scratch.

Key Action 2: Keep the Pipeline Filled: Discovery and Translation Research

There is a need to expand discovery and translational research on vaccines. The success of the current clinical candidates does not signal an end of discovery research, but rather provides novel opportunities to link fundamental research to

human studies. Current candidates are based on a very limited number, out of the potentially hundreds of antigens. It is likely that experience gained as current candidates move through clinical trials will contribute to the development of new sets of candidates in an iterative process of refinement.

Key Action 3: Facilitate Preclinical Development

There is a need to identify and assist in the development of facilities for the production of pilot lots of vaccine candidates suitable for human trials, and to ensure that these candidates are subject to appropriate tests to confirm biological potency and lack of toxicity in experimental systems.

Key Action 4: Build Capacity at Vaccine Trial Sites

Carrying out vaccine trials requires the availability of local expertise as well as baseline data in the populations who will participate in these trials. Prerequisites include baseline epidemiological information, development of community interaction programmes, development of protocols that comply with legal and ethical requirements, coordination with national regulatory authorities, local proficiency in immunological assays and optimized diagnostic procedures, and infrastructure through which the developmental vaccine will be delivered. These activities provide important opportunities for training and capacity strengthening, and require interactions with national TB control and vaccine implementation programmes.

Key Action 5: Ensure Availability of Vaccine Production Capacity/Scale-Up

The potential to scale up production of experimental vaccines to a level suitable for widespread distribution in multicentre, multinational studies is an essential factor in the selection of candidates for clinical trials. Also, it is anticipated that a new licensed vaccine would be made available at a cost that is affordable for resource-poor countries. It is likely that these demands will exceed the capacity of existing vaccine production facilities and will necessitate investment in one or more dedicated good manufacturing practice (GMP)-quality production facilities. This activity will require the development of innovative partnerships with manufacturers in developing and developed countries.

Key Action 6: Perform Clinical Trials

Evaluation of vaccine candidates requires transition through a series of clinical trials of increasing size, complexity, and cost to progressively evaluate their safety, immunogenicity, and finally efficacy. Assuring commitment of investments by collaborators in developed and developing nations is a major challenge for the global TB vaccine development community at this juncture.

Key Action 7: Provide an Enabling Infrastructure

Targeted support actions need to include assessment of the economic impact of vaccines with different performance characteristics, facilitation of international regulatory harmonization for TB vaccines and strengthening of regulatory capacity in high burden countries, identification of standard reagents and protocols to produce comparable preclinical and clinical data, identification of facilities for timely vaccine production, and preparation for accelerated access to licensed vaccines for high-burden countries.

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 (30). 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. 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 in which the Global Plan to Stop TB has identified the most pressing resource mobilization needs 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 often shy back from the administrative burden of funding these modestly sized but generally high impact activities.

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