Tuberculosis vaccines: past, present and future
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Purpose of review
The current vaccine against tuberculosis protects against severe forms of the disease in children but confers variable effectiveness against pulmonary disease. With tuberculosis eradication on the horizon new vaccines with better protection than Mycobacterium bovis bacillus Calmette–Guérin (BCG) are needed. This review will outline the most promising tuberculosis vaccine candidates from selected publications.

Recent findings
The enormous effort of the scientific community in the last 10 years has generated hundreds of tuberculosis vaccine candidates. These include sub-unit vaccines and live vaccines such as recombinant BCG and other attenuated live vaccines. Some of these are being included for the first time in phase I clinical trials.

Summary
For more than 80 years now no new tuberculosis vaccine has successfully been developed. There is now renewed optimism that vaccines superior to BCG can be developed in the coming years. The goal is to obtain a new generation of vaccines effective against more transmissible forms of tuberculosis. As a first step, good candidate vaccines able to boost BCG and improve BCG protection could be a reality in the near future. Tuberculosis vaccine candidates, able to replace the currently used BCG and make the eradication of tuberculosis feasible, can be expected in the mid-term, and live vaccines are reliable and promising candidates.

Keyword
new tuberculosis vaccines

Introduction
Mycobacterium tuberculosis is one of the most successful pathogens, such that still, in some areas of the world, tuberculosis (TB) has reached alarming proportions with a growing number of cases and deaths linked to HIV [1]. The appearance of cases of multi-drug resistant tuberculosis, some times causing outbreaks, is a serious public health problem for any attempt to control this disease [2].

The current vaccine against tuberculosis, M. bovis bacillus Calmette–Guérin (BCG), has been applied since early 1920s and is the only vaccine available for prevention of TB in humans. BCG is an attenuated live vaccine that was obtained after 230 successive passages in the laboratory between 1908 and 1921, from a pathogen strain of M. bovis. BCG confers a strong immune response, as much humoral as cellular, that is used for the treatment of bladder cancer. Recent studies demonstrated that a single dose in childhood maintains immunization for up to 50–60 years after vaccination [3]. Genomic studies demonstrated that BCG contains different deletion regions of its genome with the loss of more than 100 genes [4]. Since 1921, when BCG was used for the first time, different laboratories of the world have continued to sub-culture BCG, giving rise to the appearance of different variants such as BCG Pasteur, BCG Moscow or BCG Brazil. These variants contain different deletions that could be implicated in the degree of protection provided [5]. Since 1961 the World Health Organization (WHO) has recommended lyophilization of BCG vaccines stocks and storage at −80°C [5].

Protection studies of BCG have showed a variable effectiveness against pulmonary TB that ranges from no protection in studies in India to 70% protection in the studies of the UK Medical Research Council. This lack of protection against pulmonary TB has enormous importance from the point of view of public health as regards eradication of TB [6]. It has been hypothesized that this failure of the BCG vaccine in southern countries could be due to previous exposure to environmental mycobacteria [7]. In parallel it has been clearly demonstrated that BCG protects against serious cases of childhood TB, including meningitis and disseminated TB, and this is why BCG continues to be recommended in the vaccination calendar of the WHO in countries with a high TB incidence. Even if BCG has been demonstrated to be extremely useful and at the moment is the most utilized vaccine in the
world [8], the development of new vaccines against pulmonary tuberculosis able to replace the current vaccine BCG is an important challenge [9**]. Since the only reservoir of \textit{M. tuberculosis} is humans, the development of more effective vaccines than BCG could make TB eradication possible.

**Tuberculosis vaccine candidates: from the bench to clinical trials**

Advances in the characterization of genes and antigens of \textit{M. tuberculosis}, with the help of the genome sequences of different mycobacterial species [10], have provided insights into the tuberculosis bacillus. In addition, the current progress of mycobacterial genetics makes possible the inactivation of selected genes allowing the rational attenuation of \textit{M. tuberculosis} [11]. Basic knowledge of molecular biology of bacilli in addition to the development of new mycobacterial genetic tools put us in a better position for the construction of more effective and safer vaccines against tuberculosis than the present BCG (Fig. 1). Broadly, two approaches have been used to improve the tuberculosis vaccine: non-viable sub-unit vaccines and live vaccines. Sub-unit vaccines have been generated that can deliver immunodominant mycobacterial antigens. Both protein and DNA vaccines induce partial protection against experimental tuberculosis infection in mice. New antigen formulations, including multiple antigens or epitopes, are under investigation and it is hoped that they will afford better protection in humans. As regards live vaccines, many groups in numerous countries have embarked on the ambitious project of finding new vaccines that provide a greater level of protection than the present BCG [12,13]. A result of this basic research, the enormous effort of the scientific community in the last 10 years has generated a great number of vaccine candidates against TB to be tested in different laboratory experiments and experimental animal models [14**,15**].

**Use of animal model to test tuberculosis vaccines in preclinical trials**

The most commonly used animal model is the mouse, followed by the guinea pig. Primate models have been developed and are being used as an important test prior to clinical trials [16**]. Figure 2 presents the steps considered for tuberculosis vaccine candidates from research to clinical trials.

The advantage of the mouse model comes from the amount of reagents and genetic information available, and its logistical and economical advantages, in comparison with other models such as the guinea pig. Mice have certain tolerance to this infection; it triggers a moderate inflammatory reaction that allows the control of the bacillary concentration to a low level but without eradicating it. The commonest route of infection is intravenously, because this switches on acquired immunity very rapidly. The experimental model induced by aerosol is the most physiologically infectious route and at the same time is more aggressive for the host than intravenous administration. This is because the induction of immunity is quicker after intravenous inoculation than after aerosol. This model has demonstrated that immunity against infection is based essentially on the stimulus of a Th1 type response, that is to say, in the stimulation of CD4+T cells able to produce IFN-γ and to activate the infected macrophages [17].

Testing the protection obtained from new vaccines using the guinea pig model has become a compulsory experiment because of the extreme sensitivity that this animal has demonstrated against \textit{M. tuberculosis} inoculation, and the toxic response generated. This has allowed the comparison of different TB vaccine candidates [14]. On the other hand, the necessity to evaluate the protection of any new vaccine before carrying out human clinical trials in an experimental model physiologically closer to humans has led to the development of the primate model [18].
Sub-unit vaccine candidates

Because of safety reasons non-viable sub-unit vaccines are the first to be considered for human trials. Sub-unit vaccines have been selected by various rational and experimental approaches (Table 1). Potential tuberculosis sub-unit vaccines have been obtained using immunodominant TB antigens, as in the case of ESAT-6 which confers some degree of protection against *M. tuberculosis* in mice and recently in non-human primates [19]. Protein fusions based on ESAT-6 and antigen 85B administered with a strong adjuvant to mice to induce a strong dose-dependent immune response to the fusion proteins. This immune response was accompanied by protective immunity comparable to BCG-induced protection over a broad dose range.

Key *M. tuberculosis* antigens have been identified by analysis of host responses in healthy individuals, and purification of proteins from positive donors. Theses selected antigens have been used for the development of sub-unit vaccines against tuberculosis, such as Mtb72F, which codes for a 72 kDa polyprotein (Mtb32(C)-Mtb39-Mtb32(N)) [20].

Immunization of mice with Mtb72F protein formulated in the adjuvant AS-01B generated a comprehensive and robust immune response, eliciting strong IFN-γ and antibody responses for all three components of the polyprotein vaccine and a strong CD8(+) response directed against the Mtb32(C) epitope. Mtb72F immunization resulted in the protection of C57BL/6 mice against aerosol challenge with a virulent strain of *M. tuberculosis*. Most importantly, immunization of guinea pigs with Mtb72F resulted in prolonged survival (> 1 year) after aerosol challenge with virulent *M. tuberculosis* comparable to BCG immunization. Mtb72F in the AS-02A formulation is currently in phase I clinical trials, making it the first recombinant tuberculosis vaccine tested in humans [20].

In addition is necessary to develop a therapeutic vaccine against the latent tuberculous infection, since one third of humanity is already infected and it represents an enormous TB reservoir [21].

Boosting the bacillus Calmette–Guérin vaccine

Experiments using protein sub-units in animals previously vaccinated with BCG (BCG+), and using prime–boost protocols give very good results. These experiments used Ag85A, because it was previously demonstrated that most CD4 T cells accumulating in the lungs of memory-immune mice after challenge recognize this antigen. This vaccine strategy may have applications in the prevention of reactivation of TB in the elderly.

Heterologous prime–boost immunization strategies can evoke powerful T cell immune responses and may be of value in developing an improved tuberculosis vaccine. Enhanced immunogenicity and protective efficacy against *M. tuberculosis* has been demonstrated for BCG after boosting with a recombinant modified vaccinia virus called Ankara. The Ankara recombinant modified vaccinia virus, expressing *M. tuberculosis* Ag85A, strongly boosts BCG-induced Ag 85A specific CD4(+) and CD8(+) T cell responses in mice. Protection correlated with the induction of Ag 85A-specific, IFN-γ-secreting T cells in lung lymph nodes. This vaccine was tested for first time in humans [22].

Recombinant bacillus Calmette–Guérin as a new vaccine against tuberculosis

Recombinant BCG techniques may be useful for the development of a more effective mycobacterial vaccine than the parent BCG now in use. Various strategies have been used to develop recombinant BCG against mycobacterial diseases (Table 2). One is based on recombinant BCG producing large amounts of autologous protective antigens; these supplementary antigens are designed to enhance immunity to other BCG antigens by increasing the expression of their genes as is the case for the immunodominant TB antigens. Recombinant BCG vaccine (rBCG30) expressing and secreting the 30-kDa,

### Table 1 Tuberculosis vaccine candidates tested in humans

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Definition</th>
<th>Stage of development</th>
<th>Pharmaceutical company or research group</th>
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<tbody>
<tr>
<td>Sub-unit</td>
<td>72f Selected antigens identified from human response</td>
<td>Phase I trial ready for phase II BCG boosting strategy</td>
<td>GlaxoSmithKline (EU/TBVac/Aeras) [20]</td>
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<tr>
<td></td>
<td>85B-ESAT6 Recombinant major antigens</td>
<td>Phase I trial BCG boosting strategy</td>
<td>SSI (EU/TBVac) [19]</td>
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<tr>
<td>Viral vector</td>
<td>MVA-85A Recombinant modified vaccinia virus Ankara Ag85A</td>
<td>Phase I trial BCG boosting strategy</td>
<td>Oxford University, UK (EU/TBVac) [22]</td>
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<tr>
<td>Live vaccines</td>
<td>rBCG30 Recombinant BCG: overexpression of Ag85B</td>
<td>Phase I trial</td>
<td>(UCLA/NIH/Aeras) [23**]</td>
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BCG, bacillus Calmette–Guérin; UCLA, University of California Los Angeles; NIH, National Institutes of Health.
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Table 2 Live tuberculosis vaccine candidates in advanced preclinical testing

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<tr>
<th>Vaccines</th>
<th>Definition</th>
<th>Research group</th>
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<td>rBCG:RD1</td>
<td>Recombinant BCG RD-1 of <em>M. tuberculosis</em> introduced</td>
<td>Institut Pasteur Paris, France (EU/TBVac) [24]</td>
</tr>
<tr>
<td>rBCG-Δure-hly</td>
<td>Recombinant BCG with BCG urease gene deleted and listeriolysin of <em>Listeria monocytogenes</em> introduced</td>
<td>Max Planck Institute Berlin, Germany (EU/TBVac) [25]</td>
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<tr>
<td><em>M. tuberculosis</em> phoP mutant</td>
<td>Rational attenuated <em>M. tuberculosis</em> clinically isolated by phoP regulated virulence gene deleted</td>
<td>Zaragoza University, Spain (EU/TBVac) [26,27]</td>
</tr>
<tr>
<td><em>M. tuberculosis</em> auxotropic mutant</td>
<td>Rational attenuated <em>M. tuberculosis</em> H37Rv by lysA and panCD deletion</td>
<td>Albert Einstein College of Medicine, New York, USA (NIH) [28]</td>
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BCG, bacillus Calmette–Guérin; NIH, National Institutes of Health.

Major secreted protein of *M. tuberculosis*, also referred to as α-antigen and antigen Ag85B, is associated with better host survival after challenge than parent BCG in the highly demanding guinea pig model of pulmonary TB. Animals immunized with rBCG30 and then challenged with an aerosol of a highly virulent strain of *M. tuberculosis* survived significantly longer than animals immunized with conventional BCG [23**].

Alternatively, BCG genes that have been lost by deletion from the parent *M. bovis* strain and that are important antigens can be restored. An example is the case of ESAT-6 deleted from region RD1 of BCG [24]. Both these approaches are attractive for improving or adding antigens to BCG and could be important in conferring immunity against TB.

A second strategy involves enhancement of the relatively low intrinsic ability of BCG to induce the CD8+ T cell response. This type of recombinant BCG has been studied, and in particular whether it alters the permeability of the membranes of phagosomes in host cells. Major histocompatibility complex (MHC) class I-restricted CD8+ T cells are believed to play a major role in protection against mycobacterial infection. As BCG persists within the phagosomal space of macrophages after infection, bacterial antigens should be released from phagosomal vacuoles into the cytoplasm of host cells leading to more pronounced presentation by MHC class I. Listeriolysin of *Listeria monocytogenes* is a pore-forming sulphhydryl-activated cytolysin. It is essential for the release of *L. monocytogenes* from phagosomal vacuoles into the cytoplasm of host cells, thereby facilitating presentation of antigens by MHC class I molecules. The group of Kaufmann [25] constructed recombinant BCG secreting biologically active listeriolysin. This rBCG improves MHC class I-presentation of cophagocytosed soluble protein.

Other live vaccines

Of the six immunodominant antigens of *M. bovis* (ESAT-6, CFP10, Ag85, MPB64, MPB70, MPB83), five are either deleted from or downregulated in some or all BCG strains. RD1 is present in all BCG strains. The deletions include the immunodominant antigens ESAT-6 and CFP10, which have been shown to be important for protection against *M. tuberculosis* challenge in the guinea pig model [24]. The advantage of rational attenuated *M. tuberculosis* as a vaccine is that the hundreds of genes deleted from BCG as a consequence of the progressive adaptation of BCG strains to laboratory conditions are still present in *M. tuberculosis*.

Several studies described the development of attenuated strains of *M. tuberculosis*. An *M. tuberculosis* phoP mutant has been constructed by a single gene disruption [26] and exhibits impaired multiplication in vitro within mouse-cultured macrophages; it is also attenuated in vivo in a mouse infection model. Thus, phoP might be involved in the regulation of complex mycobacterial lipids implicated in the virulence in *M. tuberculosis* [27]. Results in an animal model make a phoP mutant a promising TB vaccine candidate [14]. Auxotrophic mutants are attenuated to different degrees and have diverse potential as vaccine candidates as assessed in animal models. Double auxotrophic mutants were recently described (Table 2) [28].

Advantages and disadvantages of live vaccine candidates

Live vaccines have been questioned because of the failure of the BCG vaccine due to some species of environmental mycobacteria that were able to block multiplication of BCG and induction of protective immunity in animal studies [7]. Evidence was provided that sensitization with environmental mycobacteria may have a direct antagonistic effect on BCG vaccination.

Recently, it was experimentally demonstrated [29*] that cross-reaction is due to shared antigens between BCG and environmental mycobacteria, such as Ag85B, but not deleted antigens of BCG such as ESAT-6 and FP10. These results strongly suggest that prior exposure to live
environmental mycobacteria primes the host immune system against mycobacterial antigens shared with BCG and that recall of this immune response on vaccination results in accelerated clearance of BCG and hence decreased protection against TB. The authors demonstrated that persistence of BCG in vivo could be markedly augmented by stable insertion of RD1, a region of difference between attenuated mycobacterial strains.

Rational attenuated *M. tuberculosis* which includes deleted regions of BCG with major antigens not shared with environmental mycobacteria will overcome the problem of the antagonistic effect of BCG to previous environmental mycobacterial immunization.

Other major issues associated with the use of live organisms, particularly safety and regulatory hurdles, need to be overcome and steps were described in a Geneva conference in 2004 [16].

**Conclusion**

For many years the discovery of new tuberculosis vaccines effective against pulmonary tuberculosis has been considered an elusive quest, but the tuberculosis vaccine field has blossomed in the last 10 years. After the Madrid Conference in March 1995 ‘Definition of a coordinated strategy towards a new TB vaccine’ organized by WHO and International Union Against Tuberculosis and Lung Disease, a joint effort was established involving diverse organizations. In Europe the EU has providing funding for a TB cluster of laboratories in several countries and in the United States of America the National Institutes of Health coordinated with Colorado State University. Recently additional funding from the Aeras Global TB Vaccine Foundation for vaccine clinical trial development has been trying to accelerate the step from bench to clinical trials.

In just the last year more than 300 articles have been published concerning TB vaccine investigation. To find a new, more effective vaccine than BCG for cases of pulmonary TB in adults is a priority of the research in TB, with the objective of preventing eradicating the disease. Developers of this new vaccine will have to keep in mind that a third of the world’s population is already infected with the TB bacillus, the HIV and TB coinfection in areas with high TB incidence and that in great number of countries the population has been previously vaccinated with BCG or are infected with TB.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).


In this updated review the host response to *M. tuberculosis* infection and tuberculosis disease is graphically described. The different TB and AIDS vaccination strategies are described as well as the regulatory issues for vaccine trials.


The authors describe the use of a guinea pig model for comparative studies of new candidate vaccines. A total of 24 vaccines were evaluated in four experiments each of a different design.


The author provides a clear description of the current progress in tuberculosis vaccine development. Description of the different TB vaccine candidates, animal models, surrogate markers and candidates moving towards clinical evaluation mainly focused on US candidates.


This presents a consensus document in order to facilitate the movement of the most promising live vaccine candidates to human trials.


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23 Horwitz MA. Recombinant BCG expressing Mycobacterium tuberculosis major extracellular proteins. Microbes Infect 2005; 7:947–954. This revision describes rBCG30, the first of the new live vaccine generation tested in humans.


29 Demangel C, Garnier T, Rosenkrands I, Cole ST. Differential effects of prior exposure to environmental mycobacteria on vaccination with Mycobacterium bovis BCG or a recombinant BCG strain expressing RD1 antigens. Infect Immun 2005; 73:2190–2196. This provides an experimental demonstration that environmental mycobacteria were able to block multiplication of BCG due to shared antigens and that this could be overcome by the construction of a new generation of live vaccines including major TB antigens not present in non-pathogenic species.
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