What's new in tuberculosis vaccines?

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Abstract Over the past 10 years, tuberculosis (TB) vaccine development has resurged as an active area of investigation. The renewed interest has been stimulated by the recognition that, although BCG is delivered to approximately 90% of all neonates globally through the Expanded Programme on Immunization, *Mycobacterium tuberculosis* continues to cause over 8 million new cases of TB and over 2 million deaths annually. Over one hundred TB vaccine candidates have been developed, using different approaches to inducing protective immunity. Candidate vaccines are typically screened in small animal models of primary TB disease for their ability to protect against a virulent strain of *M. tuberculosis*. The most promising are now beginning to enter human safety trials, marking real progress in this field for the first time in 80 years.

Keywords BCG vaccine; Mycobacterium bovis/immunology/genetics; Mycobacterium tuberculosis/immunology/genetics; Drug evaluation, Preclinical; Models, Animal; Clinical trials, Phase I; Research (*source: MeSH, NLM*).

Mots clés Vaccin BCG; Mycobacterium bovis/immunologie/génétique; Mycobacterium tuberculosis/immunologie/génétique; Evaluation préclinique médicament; Modèle animal; Essai clinique phase I; Recherche (*source: MeSH, INSERM*).

Palabras clave Vacuna BCG; Mycobacterium bovis/inmunología/genética; Mycobacterium tuberculosis/inmunología/genética; Evaluación preclínica de medicamentos; Modelos animales; Ensayos clínicos fase I; Investigación (*fuente: DeCS, BIREME*).

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Introduction

Tuberculosis (TB) vaccine discovery and development are enjoying a renaissance. This is in sharp contrast to the limited advances made in the field following the development of BCG during the first two decades of the 20th century. Today, over 70 years after its development, BCG is still the only TB vaccine available, and the achievements of TB vaccine research have been largely operational, such as expanding delivery of BCG through the Expanded Programme on Immunization and holding field trials using a variety of BCG strains in different geographical locations (*1–11*). Meta-analysis of these trials revealed a wide range of efficacy (<0% to >80%) for BCG vaccines (*12–13*), and debate continues on how to interpret these results and on the effectiveness of today's BCG vaccinations (*14–17*).

The global impact of TB is devastating, with 2–3 million deaths annually (including those of HIV-infected individuals) and over 8 million new cases. It is estimated that one-third of the world's population is infected with *M. tuberculosis* (*18*). In the early 1990s, recognition of the scale of the TB problem spurred funding agencies and scientists throughout the world to develop improved tools to diagnose, treat, and prevent TB. It is clear that more effective vaccine(s) will be key in achieving true TB control, even with early, accurate diagnosis and effective treatment. Currently, several candidate vaccines are being prepared for, or are already in, early human testing. TB programme managers and public health officials from high-burden countries should help guide the development of such vaccines by informing decisions as to which vaccines would be most helpful in the field.

The natural history of TB is complex (Fig. 1). Exposure of a healthy uninfected individual to a source case can result in primary infection with *M. tuberculosis*. In turn, this infection can develop either into primary TB disease or into a persistent, asymptomatic infection, which often remains clinically silent throughout a person's life. However, in about 10% of immunocompetent people and in 8% of HIV-positive individuals each year, a latent infection may "reactivate" and cause symptomatic TB disease. The complicated natural history of TB suggests at least three possible vaccination strategies (Table 1): one that would prevent primary infection and disease following exposure; a second that would prevent reactivation in those already infected; and a third, an immunotherapeutic adjunct to standard TB treatment, which would speed and enhance standard TB treatment in those already ill from TB. Each of these strategies has advantages and disadvantages (Table 1). Because the adjunctive therapy approach is really a treatment strategy, rather than a prevention strategy, and its literature and history deserve a careful review of their own, this approach will not be further discussed in this review.

Recent progress

Development and screening of vaccine candidates

Since 1997, over 170 vaccine candidates have been tested by Ian Orme, David McMurray, and their colleagues under a United States National Institutes of Health contract, using mice and guinea-pigs in low-dose, aerosol challenge models of primary TB disease. The candidates represent four basic vaccine types. The first, subunit vaccines, consist of one or

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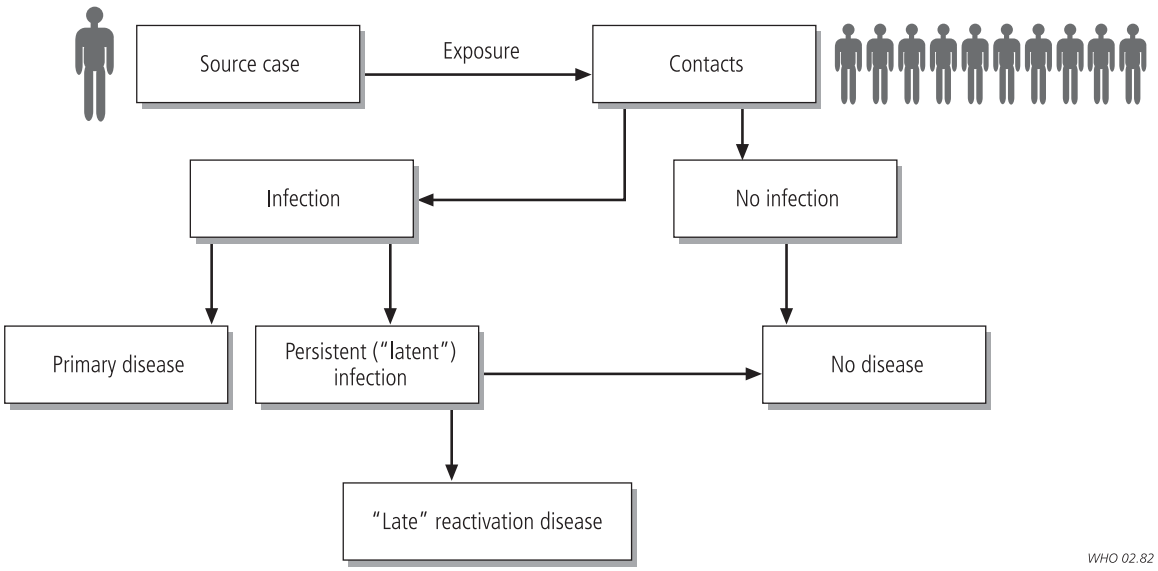
Fig. 1. **Natural history of tuberculosis (TB)**



more mycobacterial components believed to induce protective immunity, and represent over 50% of all candidates tested under the contract. The majority of subunit vaccine candidates tested are composed of protein subunits, but a few use lipid or carbohydrate subunits. The second type is naked DNA vaccines. The third is vaccines based on live, attenuated mycobacteria, including recombinant BCGs (rBCGs) expressing immunodominant antigens and/or cytokines, attenuated strains of *M. tuberculosis,* and nonpathogenic mycobacteria (e.g. *M. vaccae*, *M. smegmatis*, *M. microti*, and *M. habana*). The fourth type is vaccines based on live, attenuated, nonmycobacterial vectors, such as *Salmonella* or vaccinia virus. A European consortium funded by the European Commission (*19*) and a small number of other laboratories around the world under WHO's aegis have also screened limited numbers of potential candidates in similar animal models.

Why has there been such a plethora of candidate vaccines developed, what have we learned from them, and where are they leading the TB vaccine field? The remainder of this review will explore these questions.

Lessons from screening in animal models

A number of general lessons have been learned from screening potential vaccine candidates in small animal models. First, BCGs (a number of different strains) protect relatively well and consistently in these models. This is to be expected as the low-dose, aerosol challenge models in mice and guinea-pigs, currently considered the standard for such experiments, most closely mimic primary TB disease in a naive host. This is also the type of human TB against which BCG works best (i.e. protection of infants and young children — immunologically naive hosts — from primary TB disease).

Second, only a small number of candidate vaccines tested in these animal models have protected as well as BCG. Significantly, however, a subset of them has provided even better protection than control BCGs (as measured by number of colony forming units in lung, liver, and spleen, and clinical features such as weight change and survival). These candidates are now being prepared for safety and immunogenicity testing in humans.

Third, these results have highlighted the need to develop animal models that better mimic the conditions of most human disease — i.e. hosts that have already been immunized with BCG, exposed to environmental mycobacteria, and/or are persistently infected with *M. tuberculosis*. Attempts to develop and standardize such models are underway (I. Orme & D. McMurray, personal communication, 2002).

Fourth, short-term protection studies in small animal models provide limited information about the potential of a vaccine candidate. Promising candidate vaccines should also be evaluated for longer-term protection (measured by survival, clinical evaluation, and pathology).

Fifth, the predictive ability, and therefore the ultimate value of these animal models, will only be clear once human and animal trials of the candidate vaccines can be compared. The most promising candidates from the screening studies should therefore enter human trials as soon as feasible. The first human safety and immunogenicity study of a novel candidate has already begun and several more are expected to begin within the next 6–12 months.

Related research areas: genomics and postgenomics

Not only has TB vaccine development undergone a renaissance in recent years, but TB research overall has grown dramatically since the early 1990s (*20*). The United States National Institutes of Health, the largest public funder of TB research globally, has increased its support for this field from US$ 3.4 million in 1990 to approximately US$ 80 million in 2000. This investment has led to new insights into the biology and biochemistry of *M. tuberculosis*; the pathogenesis of disease; underlying mechanisms of virulence and persistence; and insight into the host immune response in TB. All of these areas require more work, but each has contributed to new hypotheses about TB virulence and protection and to the development of candidate vaccines.

Table 1. **Strategies for developing tuberculosis (TB) vaccines**

Strategy	Goal	Advantages	Disadvantages
Preinfection	• Prevent infection and/or primary disease, persistence, and reactivation.	• Given to neonates — delivery system in place. • No concern about vaccinating tuberculin positives. • Animal models available.	• Major morbidity and mortality burden is in adults. • May be unrealistic to vaccinate neonates against adult TB. • Large, long efficacy trials.
Postinfection	• Prevent progression in recently exposed individuals. • Prevent reactivation in persistently infected individuals.	• Targets 1/3 of world population already infected. • Would address burden of disease — i.e. adult pulmonary TB. • Efficacy trials could be shorter and smaller (in high-risk adult populations).	• Few known examples of vaccines that work postexposure (rabies, tetanus; some preliminary evidence for smallpox and hepatitis B).
Adjunctive immunotherapy (not reviewed in this article)	• Treat patients with active TB to improve treatment efficacy and/or shorten the course of chemotherapy.	• Uses vaccine in conjunction with conventional chemotherapy. Might shorten treatment duration. • Trials similar to conventional drug trials; could be conducted in accessible, well-defined populations.	• Feasibility not established. • Potential for exacerbation of disease by immune responses must be determined.

Perhaps the greatest stimulus to vaccine development has come from the complete genomic sequencing of two strains of *M. tuberculosis* (*21, 22*), as well as of *M. bovis*, *M. smegmatis*, *M. avium, M. paratuberculosis, M. ulcerans* and *M. leprae* (*23, 24*) and the development of new techniques for the easy molecular manipulation of mycobacterial genomes (*25–27*). In addition, a new consortium is working to solve the three-dimensional structure of about 400 *M. tuberculosis* proteins over five years (*28*). This should provide valuable insights into virulence factors and other key proteins, and help identify targets for TB drug discovery.

Improving BCG

An alternative strategy being explored by a small number of investigators involves attempts to improve BCG, for example by testing its efficacy at lower doses and in a prime-boost protocol. This strategy is based, in part, on data from a deer infection model indicating that double vaccinations delivered as a prime, followed in a few weeks by a booster dose, are more efficacious than single vaccinations in protecting against infection and disease (*29, 30*). Hoft and colleagues are investigating the human immune response to BCG when delivered in various ways, to see whether BCG might be made more efficacious by altering its delivery method (*31, 32*). Oral delivery, the original method of administering BCG, for example, might stimulate mucosal immunity better than the currently favoured intradermal route.

Still other investigators are attempting to improve on BCG by using prime-boost strategies that combine BCG (as either the prime or boost) with a novel candidate vaccine. The hope is that the combination will act additively or even synergistically to improve the effectiveness of the BCG vaccine (*33–35*). If successful, strategies that modify current BCG use could lead to significantly faster improvements in TB vaccination effectiveness than having to license and deliver an entirely new vaccine.

Testing novel vaccine candidates in humans

To this author's knowledge, the first novel candidate to enter Phase 1 trials for safety and immunogenicity in the current period is an immunodominant protective antigen, Ag85A, from *M. tuberculosis* expressed in a replication-deficient strain of vaccinia virus (MVA-Ag85A) (H. McShane & A. Hill, personal communication, 2002). McShane and colleagues are testing MVA-Ag85A in a small number of tuberculin skin test-positive individuals and ultimately hope to investigate the safety, immunogenicity, and efficacy of a BCG prime/MVA-Ag85A boost strategy in both skin test-positive and -negative individuals. In a similar prime-boost strategy, they also plan to test a recombinant poxvirus, FP9, expressing Ag85A.

Other candidates being readied for human testing in the next 6 months–2 years include: a recombinant BCG over-expressing Ag85B (M. Horwitz, personal communication, 2002), in a collaborative effort with the Sequella Global TB Foundation and the United States National Institute of Allergy and Infectious Disease; a subunit vaccine composed of *M. tuberculosis*-derived immunodominant fusion proteins, Ag72f+/-Ag85 (S. Reed, personal communication, 2002); a multi-epitope subunit vaccine/adjuvant combination developed by InterCell Corporation; and, potentially, attenuated mutants of *M. tuberculosis* being developed at Albert Einstein College of Medicine, New York, USA, and the Howard Hughes Medical Institute, New York, USA, by William Jacobs Jr (W.R. Jacobs Jr, personal communication, 2002). These attenuated strains of *M. tuberculosis* lack the ability to make key amino acids or vitamins and cannot survive in the host for long. The hope is this will render them safe for use, even in immunocompromised individuals.

Jacobs and others have also recreated in *M. tuberculosis* what is believed to be the original attenuating mutation of *M. bovis*, the RD1 deletion, that led to the creation of BCG (*36*; W.R. Jacobs Jr, personal communication, 2002; D. Sherman, personal communication, 2002). The RD1 deletion in *M. tuberculosis* will be used to test the hypothesis that such a strain will be attenuated, but persist long enough in the human

host to stimulate a protective immune response, perhaps even stronger than that induced by BCG. The fundamental basis of *M. tuberculosis* virulence is being further explored by comparing the genomes of virulent (disease-causing) to those of non-virulent mycobacteria, as well as by comparing various strains of BCG to each other and to virulent mycobacteria.

M. vaccae, an environmental mycobacterium, is also being tested in human trials. A heat-inactivated form is being investigated as a preventive vaccine in a trial of a five-dose regimen in HIV-positive individuals (*37*).

Remaining questions, ongoing challenges

These exciting advances, which utilize modern techniques of vaccinology, are counterbalanced by ongoing challenges and remaining questions, some of which are outlined here. First, what are the human and mycobacterial mechanisms that enable *M. tuberculosis* to persist asymptomatically for long periods in the human host, then reactivate to cause disease and further transmission? Answers should come with progress in modelling the natural history of *M. tuberculosis* infection and disease, and through the application of functional genomics (*38–40*) and proteomics (*41, 42*) to the identification of mRNAs and proteins involved in host–bacterial interactions.

Second, what constitutes the human protective immune response to *M. tuberculosis* and which element(s) could be used as surrogate markers of protective efficacy to speed and simplify human vaccine trials? Studies in South Africa (supported by the Sequella Global Tuberculosis Foundation and GlaxoSmithKline) and Uganda (supported by the United States National Institute for Allergy and Infectious Diseases under its Tuberculosis Research Unit (TBRU)), are trying to determine the key elements of the human protective immune response to *M. tuberculosis* infection. The Sequella Global TB Foundation is sponsoring a randomized-controlled BCG vaccine trial, conducted by investigators from the University of Cape Town, to compare percutaneous and intradermal administration of Japanese 172 BCG vaccine administered at birth. In a nested case-control study to identify immune correlates of vaccine protection or failure, specimens from TB patients identified through the surveillance activities of the BCG trial will be compared with specimens from suitably matched, healthy controls who are identified as resident in a household containing an individual with infectious TB (L. Geiter, personal communication, 2002).

Research teams funded by GlaxoSmithKline's Action TB Initiative are using differential gene expression technology to identify potential biomarkers of immune status. At the University of Stellenbosch Faculty of Health Sciences, South Africa, the strategy is to compare gene expression in groups of individuals with different susceptibilities to TB. Studies at the London School of Hygiene and Tropical Medicine, under the Action TB Initiative, are aimed at identifying genes expressed in cytotoxic CD8+ T-cells (K. Duncan, personal communication, 2002). Under the TBRU, investigators from Makerere University in Kampala, Uganda, and Case Western Reserve University, Cleveland, USA, are conducting a household contact study that will follow index cases during and after treatment. The goals are to determine immunological, microbiological, and clinical risk factors for clinical progression of the disease, as well as for mycobacterial reactivation and reinfection in subjects who relapse. The investigators will also follow household contacts of index cases to determine host immunological factors associated with tuberculin skin test conversion or development of primary infection. This study represents a follow-up to an earlier TBRU household contact study in Uganda, which indicated that interferon production by *M. tuberculosis*-stimulated whole blood cells served as an imperfect, but best available, correlate of human protective immunity (*43*).

The first human efficacy trials of novel candidates, as well as further investigation of the human immune response to BCG, should help confirm the key immune factors identified in such studies and enable their use as correlates of protection to speed and simplify future vaccine trials.

The third challenge is to identify the role(s) of coinfections and comorbidities in TB (e.g. environmental mycobacteria, helminths, and viral infections including HIV). How can we safely and effectively vaccinate populations with high levels of HIV infection? More cross-fertilization and collaboration with investigators in these areas of infectious disease research and public health are needed to help answer these questions. Success in tackling these three challenges would enable TB vaccines to be designed faster and more rationally than is currently possible.

Finally, TB vaccine developers will also need to address important ethical issues as they move towards human trials and vaccine delivery. One issue is the ethics of withholding BCG vaccine during clinical trials. BCG appears to provide significant protection against extrapulmonary TB in young children in many, if not all, settings where it is routinely used (*13*), and also appears to provide some protection against leprosy (*44*). Withdrawing its use in clinical trials is thus difficult to rationalize until a new vaccine has proved to be as safe and effective. One exception to this may be trials of a recombinant BCG candidate, but careful discussion of even this scenario will be necessary in planning human studies.

A second ethical issue is how to test novel vaccines safely in populations where HIV infection is prevalent, since these are often the same populations with high rates of *M. tuberculosis* infection. A third issue concerns the obligations that vaccine developers have to populations in resource-poor settings that have participated in clinical testing of a vaccine candidate, should the candidate prove efficacious. Finally, how can the public sector and other interested parties help ensure that high-burden, low-resource countries have access to an efficacious vaccine once it is developed? Governments, populations, and health care providers from around the world must be involved in these discussions from early in the vaccine development process.

Conclusion

A recent modelling exercise demonstrated that more than one billion people would save more than US$ 25 in medical costs if they received a 75%-effective vaccine of 10 years' duration (*45*). Many more, of course, would benefit from the resulting prevention of lost productivity and improved quality of life. Ultimate control of TB in both industrialized and resource-limited countries will require not only our best efforts to identify and treat TB whenever and wherever it occurs, but also optimal approaches to preventing disease and transmission by delivering efficacious TB vaccines. Recent advances may at last be bringing this previously elusive goal within our reach. ■

Conflicts of interest: none declared.

Résumé

Vaccins antituberculeux : quoi de neuf ?

Depuis dix ans, le développement de vaccins antituberculeux fait de nouveau l'objet de recherches actives. Ce regain d'intérêt a été suscité par le fait que, malgré l'administration du BCG à environ 90 % des nouveau-nés dans le monde grâce au Programme élargi de vaccination, *Mycobacterium tuberculosis* continue à provoquer chaque année plus de 8 millions de nouveaux cas et plus de 2 millions de décès. Plus de 100 vaccins candidats faisant appel à diverses approches pour induire une immunité protectrice ont été préparés. Ces vaccins expérimentaux subissent en général un premier tri sur de petits modèles animaux de tuberculose primaire pour tester leur pouvoir protecteur contre une souche virulente de *M. tuberculosis*. Les plus prometteurs d'entre eux commencent maintenant à faire l'objet d'essais d'innocuité chez l'homme, ce qui constitue la première véritable avancée dans ce domaine depuis 80 ans.

Resumen

Vacunas contra la tuberculosis: novedades

A lo largo de la última década el desarrollo de una vacuna contra la tuberculosis ha resurgido como una activa línea de investigación. Este renovado interés se ha visto estimulado por el reconocimiento del hecho de que, pese a que el BCG se administra a aproximadamente al 90% de todos los recién nacidos a nivel mundial en el marco del Programa Ampliado de Inmunización, *Mycobacterium tuberculosis* sigue causando más de 8 millones de casos nuevos de tuberculosis y más de 2 millones de defunciones cada año. Se han desarrollado más de 100 vacunas experimentales contra la tuberculosis, empleando distintas tácticas para inducir inmunidad protectora. Estas vacunas experimentales suelen ensayarse en pequeños animales utilizados como modelos de la infección primaria, para determinar su capacidad de protección frente a una cepa virulenta de *M. tuberculosis.* Las vacunas más prometedoras se han empezado a utilizar ya en ensayos de seguridad en la especie humana, lo que constituye un auténtico progreso en este campo por primera vez en 80 años.

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