Clinical Medicine 2012, Vol 12, No 6: s17–s20

Global progress in tuberculosis vaccine development

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Introduction

Progress is being made in the global control of tuberculosis (TB), with mortality nearly a third lower than in 1990. Nevertheless, 90 years after the development of the *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) vaccine, TB continues to be one of the foremost global health problems, with 1.4 million deaths from TB in 2010. More than 2 billion people are estimated to be latently infected with the causative organism *Mycobacterium tuberculosis*, and these people carry a 10% lifetime risk of progressing to active TB, therefore creating a vast reservoir of potential disease. Coinfection with human immunodeficiency virus (HIV) greatly increases this risk to 10% per year. A quarter of the 1.8 million deaths from HIV in 2010 were caused by TB, with most of these in subSaharan Africa, where the rising incidence of TB has not yet reversed.[1]

Several reasons explain why TB has not yet been adequately controlled. First, diagnostic technology is inadequate, making the identification of cases of latent *M tuberculosis* infection and infectious TB disease challenging. Second, antituberculous drug treatment is long and complicated by the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M tuberculosis*. Encouragingly, a number of new antituberculous agents have recently entered phase 2 and 3 trials almost 50 years after the approval of rifampicin, the last new drug against TB (reviewed in Ref 2). Third, evidence gathered from 60 years of controlled trials with the BCG vaccine shows that the protective efficacy of the vaccine against TB is highly variable.

New tools are urgently needed to address these problems. Despite progress in the diagnosis and treatment of TB, preventative strategies, namely effective vaccination, are desirable and cost effective. New vaccines are a key element of the World Health Organization's (WHO) *Global plan to stop TB 2011–2015*.[3] This plan endorses the Stop TB Partnership's targets, which are to reduce the global burden of TB disease (deaths and prevalence) by 50% relative to 1990 levels by 2015 and to reduce the global incidence of TB disease to fewer than one per million population by 2050, thus eliminating TB as a public health problem.[3]

Bacille Calmette-Guérin vaccine

The BCG vaccine affords highly variable protection against TB depending on geography, age and type of TB disease. Efficacy against pulmonary TB ranges from 0% to 80%, with the lowest estimates in tropical regions in which TB is endemic. This is a major obstacle in global control of TB because most adult TB disease is of the pulmonary type and these patients are the predominant source of *M tuberculosis* transmission. Children are much more susceptible than adults to extrapulmonary forms of TB disease, especially disseminated and meningeal TB. The efficacy of BCG in preventing these severe forms of TB is about 70%.[4] Revaccination of older children previously immunised with the BCG vaccine at birth does not confer substantial additional protection.[5]

The lack of consistent protection against pulmonary disease has led many developed countries that have a low burden of TB to refrain from (eg, the USA) or discontinue (eg, the UK) routine BCG vaccination. Despite these limitations, the WHO recommends BCG vaccination as soon as possible after birth in high-burden countries in view of the beneficial effect of the vaccine against severe forms of childhood TB. The exception is infants known to be infected with HIV, in whom the risk of disseminated BCG disease outweighs the benefit. The BCG vaccine is thus given to about three quarters of infants worldwide as part of the WHO's Expanded Programme on Immunization (EPI).

A number of theories have been put forward to explain the wide disparity in efficacy of the BCG vaccine. Increased exposure to non-tuberculous mycobacteria in tropical compared with temperate regions may either 'mask' the beneficial effect of the BCG vaccine (by generating substantial antituberculous immune responses prior to BCG vaccination) or 'block' the effect of the vaccine (by inhibiting BCG activity, thereby impairing the protective immunity induced by vaccination).[6,7] Other factors including host genetics, differences in BCG strains and coinfection with helminths, as well as differences in the nutritional status of trial participants, may also contribute to this geographical variation in efficacy.[8]

Inducing cell-mediated immunity

The BCG vaccine is the only licensed vaccine today that is thought to work primarily by inducing T-cell responses. Such responses are crucial to protection against TB, as *M tuberculosis* is predominantly an intracellular pathogen that is not accessible to humoral attack. The essential role of CD4-positive T cells in protection is best illustrated in the HIV-infected population, in whom disease susceptibility increases as CD4-positive T-cell counts decline. CD8-positive T cells are also likely to be important, perhaps in maintaining latent *M tuberculosis* infection.[9] The cytokines interferon gamma (IFNγ) and tumour necrosis factor (TNF) are also essential. Evidence for this comes from both gene knockout animal experiments and clinical observations. Inherited

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mutations of the IFNγ pathway increase susceptibility to mycobacterial disease, including TB.[10] Patients with Crohn's disease and rheumatoid arthritis treated with anti-TNF monoclonal antibodies have an increased risk of reactivation of latent *M tuberculosis* infection. In addition, interleukin 2 (IL-2), which is important for induction of central immune memory, as well as gamma delta T cells, CD1-positive natural killer T cells and T helper 17 (Th17) cells, may play a role in protection. These various aspects of cell-mediated immunity are necessary for protection from TB; however, they may not be sufficient.

A universally effective vaccine against TB will need to induce strong and durable T-cell immunity. In order to retain the beneficial effects of the BCG vaccine in childhood, it is widely believed that the BCG vaccine should be part of any novel TB vaccine regimen. A number of the candidate TB vaccines currently in clinical development are therefore designed as subunit vaccines to boost the BCG vaccine. This heterologous prime–boost approach, in which the boost vaccine contains antigens in common with the priming vaccine, is employed by the two most clinically advanced candidates. A second approach is to replace the current BCG vaccine with an improved BCG vaccine. It may also be attractive to combine these strategies and boost an improved BCG vaccine; however, this still needs proof of concept and would ultimately require two new vaccines to be developed to licensure. Importantly, the BCG vaccine is contraindicated in HIV-infected individuals due to the risk of disseminated BCG disease, so other strategies may be required in this population.

New vaccines – improving the BCG vaccine

A number of groups have attempted to improve the BCG vaccine by modifying its virulence and antigenicity through genetic manipulation – for example, by replacing genes lost during the attenuation of the original *M bovis*, modifying mechanisms of immune evasion or overexpressing immunodominant proteins. Of the three recombinant BCG vaccines that have entered phase 1 trials, two (rBCG-30 and AERAS-422) are not currently in active development. The third, VPM1002 (previously called ΔureC hly+), which comprises a BCG Prague strain that expresses membrane-perforating listeriolysin of *Listeria monocytogenes* and is lacking urease C, has gone forward to phase 1/2a trials in South Africa. The absence of urease C permits acidification of the phagosome to enable enhanced membrane-perforating action of the overexpressed listeriolysin. Antigenic fragments are able to leak into the cytosol, promoting class I antigen presentation and CD8-positive T-cell induction.

An alternative strategy is to replace the BCG vaccine with a different attenuated mycobacterial species. Recombinant *M smegmatis* and *M microti*, as well as two attenuated strains of *M tuberculosis* (one recombinant live auxotrophic and one gene-deleted), are currently in development but are yet to reach clinical trials.

New vaccines – boosting the BCG vaccine

All candidate boost vaccines currently in development are subunit vaccines, either adjuvanted protein vaccines or viral vector vaccines. The latter consist of recombinant viruses expressing the immunodominant antigen(s). Table 1 summarises the seven candidate boost vaccines currently in clinical development.

M72 is a recombinant protein vaccine consisting of the 32 kDa and 39 kDa antigens from *M tuberculosis*, which are also expressed in the BCG vaccine, delivered with a proprietary GlaxoSmithKline (GSK) Biologicals' liposomal-based adjuvant formulation called AS01. This is currently being evaluated in phase 2a trials in South Africa and the Gambia. An adjuvanted fusion protein of antigen 85B and early secreted antigenic target-6 (ESAT-6) called Hybrid-1 is being developed by Statens Serum Institut (SSI). The potential confounding effect of an ESAT-6-containing vaccine on the diagnostic tests for latent TB infection has been avoided in the newer SSI vaccine HyVac-4, in which ESAT-6 is replaced by antigen TB 10.4. A third SSI vaccine called Hybrid-56, which contains latency-associated protein Rv2660c, as well as the early expressed proteins 85B and ESAT6, has entered phase 1 trials.

Human adenoviruses and modified vaccinia virus Ankara (MVA) are increasingly being used as vectors for recombinant T-cell vaccine constructs, and these two viruses are the basis for the two most advanced new TB vaccine candidates: AERAS-402 and MVA85A. AERAS-402 is a replication-incompetent human adenovirus serotype 35 (AdHu35) that expresses the three

Table 1. Summary of new vaccines against tuberculosis undergoing clinical development to boost the Bacille Calmette-Guérin (BCG) vaccine.

Type of vaccine	Product	Description	Current development phase
Recombinant protein	Hybrid–1 + CAF01	Antigens 85B and ESAT-6 plus adjuvant CAF01	1
	HyVac 4/AERAS-404 + IC31	Antigens 85B and TB10.4 plus adjuvant IC31	1
	Hybrid 56 + IC31	Antigens 85B, ESAT-6 and Rv2660 plus adjuvant IC31	1
	M72 + AS01	Antigens Rv1196 and Rv0125 (32kDa and 39kDa antigens) plus adjuvant AS02	2a
	Hybrid-1 + IC31	Antigens 85B and ESAT-6 plus adjuvant IC31	2a
Viral vectored	AERAS-402/Crucell Ad35	Adenovirus 35 vector expressing antigens 85A, 85B and TB10.4	2b
	MVA85A	MVA virus vector expressing 85A	2b

CAF = cationic adjuvant formulation; IC = Intercell; ESAT-6 = early secretory antigenic target 6; AS = adjuvant system; Ad = adenovirus; MVA = modified vaccinia virus Ankara

antigens 85A, 85B and TB 10.4 in a fusion protein. AdHu35 has lower background seroprevalence than other adenoviral strains, which is an important consideration, because pre-existing human immunity induced by natural exposure to adenoviruses may affect vaccine utility.[11] AERAS-402 is a potent inducer of antigen-specific, CD8-positive T-cell responses and is currently in a phase 2a trial in HIV-infected adults in South Africa and a phase 2b trial in BCG-vaccinated infants in South Africa, Mozambique and Kenya.

Development of MVA85A

MVA is an attenuated strain of vaccinia virus, which was used as the smallpox vaccine that was given to more than 100,000 people during the smallpox eradication campaign. Antigen 85A is an immunodominant antigen from *M tuberculosis*, which is conserved in the BCG vaccine and all other mycobacteria. MVA85A is a live, non-replicating, recombinant strain of MVA that expresses antigen 85A.

Preclinical testing is an essential step in the development of a new TB vaccine. There are four main animal models of TB: mice, guinea pigs, non-human primates and cattle. Boosting the BCG vaccine with MVA85A can improve protective efficacy compared with the BCG vaccine alone in all four of these animal models.[12–15] MVA85A entered phase 1 clinical trials in September 2002. Safety is the primary outcome in these trials, and a particular concern in the field of TB vaccines was the potential for a Koch reaction – an immunopathological phenomenon potentially triggered when strong antimycobacterial responses are induced in individuals previously sensitised, or infected with, mycobacteria. The first-in-man trial of MVA85A was therefore undertaken in healthy, BCG vaccine-naïve, *M tuberculosis*-uninfected adults in the UK. Safety was subsequently evaluated in BCG vaccine-primed adults and then adults latently infected with *M tuberculosis* – first in the UK and then in Africa, where the mycobacterial load from environmental exposure is greater. A similar stepwise method has been applied for age de-escalation (moving from adults to adolescents to children and then infants) and dose-finding trials. More than 2,000 individuals (including 47 latently infected with *M tuberculosis* and 108 infected with HIV) have now received MVA85A in 19 clinical trials. MVA85A has so far been safe and well tolerated, with no symptoms, signs or radiological evidence of immunopathology in any trials to date.

The secondary outcome, which is immunogenicity, is measured by an *ex vivo* IFNγ ELISpot assay. This detects the number of antigen-specific, IFNγ-secreting T cells in the blood of vaccinated individuals. Boosting the BCG vaccine with MVA85A induces strong and durable immune responses that are higher than those induced by the BCG vaccine or MVA85A alone.[16] This finding mirrors the data from preclinical challenge models and demonstrates the potential added value of the heterologous prime–boost approach. More detailed immunological assessment is conducted on samples from the clinical trials using multiparameter flow cytometry.

The principal target populations for MVA85A are infants vaccinated with the BCG vaccine at birth, adolescents and young adults in whom the effects of childhood BCG are waning, and adults living with HIV. It has therefore been important to conduct phase 1/2a trials in each of these groups. MVA85A is safe and induces durable immunity in South African adolescents, children and infants and in *M tuberculosis*- and HIV-infected subjects. No clinically significant effect of MVA85A on HIV viral load or CD4-positive T-cell count has been observed.[17]

Another important issue for any new infant vaccine is the potential effect of coadministration with vaccines in the current expanded programme on immunisation (EPI). A clinical trial in the Gambia showed no effect of MVA85A on the existing licensed vaccines in the EPI but did show a reduction in the immunogenicity of MVA85A.[18] The clinical significance of this reduced immunogenicity is unclear, as the coadministered group still developed antigen 85A-specific immune responses. Such interference may be overcome by using a higher dose, as a relatively low dose was used in this trial. Finally, this may be a generic effect for many T cell-inducing vaccines, and further studies are needed to assess this.

Although MVA85A seems to be inducing a desirable cellular immune response, the only way to evaluate vaccine-induced protection against TB is through efficacy trials. Demonstration of preclinical efficacy by *M tuberculosis* challenge is an important proof of concept before moving into clinical trials, yet we do not know if these animal models are predictive of human efficacy. Furthermore, there are no validated immunological correlates of protection with which to select vaccine candidates. Efficacy trials must take place in a region of high TB incidence and by groups with experience and knowledge to conduct clinical trials. MVA85A is currently in two phase 2b efficacy trials: the first, in the Western Cape of South Africa, has now fully enrolled 2,797 BCG-vaccinated infants who were randomised at 18–26 weeks of age to receive MVA85A or placebo (NCT00953927); the second, a two-site trial in Senegal and South Africa, is currently enrolling 1,400 HIV-infected adults randomised to two doses of MVA85A or placebo (NCT01151189).

The infant efficacy trial is due to be unblinded in late 2012. For the first time, protection (or lack of protection) afforded by a new TB vaccine will be analysed alongside markers of the immune response to establish which, if any, are correlates of protection (or susceptibility). The findings could also better inform the usefulness of our current animal models. Bridging the knowledge gap between protection and immunity is undoubtedly necessary to achieve the overall aim of tackling the global burden of TB disease.

Summary

The BCG vaccine alone is an inadequate vaccine platform to achieve the targets of the WHO's *Global plan to stop TB* previously noted.[3] Significant progress has been made in TB vaccinology in the last decade. More than a dozen new vaccine candidates designed to replace, improve on or boost the BCG vaccine

have entered the clinical development pipeline. Many more novel vaccine candidates are undergoing preclinical evaluation. Through advanced translational research and international collaborative efforts, two of these candidates have now reached phase 2b efficacy trials. Whether or not these vaccine candidates will protect against TB remains to be seen, nevertheless the findings from these trials are expected to bring us closer to a universally effective TB vaccination programme.

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