Mycobacterium bovis BCG and New Vaccines for the Prevention of Tuberculosis

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ABSTRACT Tuberculosis infects millions of people worldwide and remains a leading global killer despite widespread neonatal administration of the tuberculosis vaccine, bacillus Calmette-Guérin (BCG). BCG has clear and sustained efficacy, but after 10 years, its efficacy appears to wane, at least in some populations. Fortunately, there are many new tuberculosis vaccines in development today, some in advanced stages of clinical trial testing. Here we review the epidemiological need for tuberculosis vaccination, including evolving standards for administration to at risk individuals in developing countries. We also examine proven sources of immune protection from tuberculosis, which to date have exclusively involved natural or vaccine exposure to whole cell mycobacteria. After summarizing evidence for the use and efficacy of BCG, we detail the most promising new candidate vaccines against tuberculosis. The global need for a new tuberculosis vaccine is acute and huge, but clinical trials to be completed in the coming few years are likely either to identify a new tuberculosis vaccine or to substantially reframe how we understand immune protection from this historical scourge.

INTRODUCTION

A critical component of global tuberculosis control is the development of more effective immunization strategies. Several tuberculosis vaccines have been shown to reduce the risk of disease and death due to tuberculosis in humans, but only one is used in global immunization programs: Mycobacterium bovis bacillus Calmette-Guérin (BCG). BCG is an attenuated live vaccine administered at birth to children in most countries where tuberculosis is endemic. BCG has been the most widely administered vaccine in the world, with an estimated three billion doses administered to date (1). BCG has likely reduced the burden of tuberculosis in many areas, but it has numerous limitations. These limitations, together with the continuation of the global tuberculosis epidemic, have made the development of a more effective vaccine against tuberculosis a major international public health priority (2, 3).

The development of new tuberculosis vaccines has been informed by evolving data on the natural history of tuberculosis, by new data on the immunology of infection with and immunization against Mycobacterium tuberculosis, by reanalysis of the role of nontuberculous mycobacteria (NTM) in protection against tuberculosis, by a clearer understanding of the benefits and risks of BCG, and by molecular techniques that have permitted identification of immunodominant antigens of M. tuberculosis and new methods of antigen delivery.

GLOBAL TUBERCULOSIS IN THE BCG ERA

Approximately one-third of the world’s population is infected with M. tuberculosis, with an annual per-person incidence of new infections in the developing world of...
1 to 5%. In 2014, tuberculosis sickened over 9 million people and killed 1.5 million, among them 140,000 children. Tuberculosis is a leading cause of death among people with HIV in particular, and it disproportionately affects people in developing countries. Despite the availability of potent drugs to treat tuberculosis, the incidence of drug resistance is growing, with 480,000 people with drug-resistant tuberculosis reported in 2014 (4). For these many reasons, improving vaccine-based prevention of tuberculosis disease is a key global health priority.

**MANIFESTATIONS OF INFECTION AND DISEASE DUE TO M. TUBERCULOSIS: IMPLICATIONS FOR PREVENTIVE IMMUNIZATION**

**Initial Infection**

*M. tuberculosis* infects humans when humans inhale droplet nuclei containing viable organisms, which reach the alveoli of a nonimmune host. An initial bacteremia distributes organisms to other organ sites and to other areas of the lung. Approximately 10% of infected persons subsequently develop active disease: 5% who develop progressive primary disease within the first 2 years of infection and 5% who develop reactivation disease in the lungs or another organ site years later. The likelihood of developing primary or reactivation tuberculosis varies widely, however, with substantial incidence rates in vulnerable populations such as children, the elderly, and immunocompromised individuals. The ideal tuberculosis vaccine would prevent both initial tuberculosis infection and primary or reactivation disease in healthy hosts as well as in particularly vulnerable populations.

**Tuberculosis in Children**

Most new infections and approximately 10% of all cases of disease due to *M. tuberculosis* worldwide occur in children (5). Most infections in neonates result from exposure of the infant to an adult with active pulmonary disease soon after delivery (6). Primary infection in children is often asymptomatic, although approximately 40% of infants and 15% of children under the age of 5 develop active disease within 1 to 2 years of infection. The protean manifestations of childhood tuberculosis, the difficulty in diagnosis, and the high mortality rate are all arguments for a more effective vaccination strategy to prevent tuberculosis in children.

**Tuberculosis in Older Children and Adults**

After infancy and early childhood, progression of latent infection to active disease is most common in early adulthood (15 to 25 years of age) and in the elderly. While infection in children from ages 5 to 14 years may occur, this age group is relatively resistant to disease progression. Infection after the age of 15 progresses to active disease in 5% in the first 2 years and then in 5% over the remainder of life. Infection after age 35 is less likely to progress to active disease (7) and has a better prognosis. New infection or reactivation in the elderly tends to progress to active disease in a manner similar to that in adolescents and has a high mortality rate (8). The high rate of disease and high mortality rate in the elderly emphasize the importance of a vaccine strategy that produces durable immunity.

**Tuberculosis in the Elderly**

The risk of reactivation tuberculosis increases with age, likely through waning cellular immunity. Furthermore, the likelihood of institutionalization increases with age, and institutionalization carries with it additional risk of disease, likely related to both infirmity and intra-institutional transmission of tuberculosis. For instance, one Arkansas study showed tuberculosis case rates of 20/100,000 for the general population, 60/100,000 for individuals over age 65 who lived at home, and 234/100,000 for individuals over age 65 who lived in a nursing home (9). In the United States, the incidence of reactivation tuberculosis among the elderly has fallen dramatically, likely due to the disappearance of untreated old tuberculosis (10).

**Tuberculosis and HIV Infection**

Among the 1.4 million people who died of tuberculosis disease in 2014, approximately 400,000 were HIV infected (4). Like with other forms of immunodeficiency, the risk of progression from latent tuberculosis infection to active tuberculosis is heightened during HIV infection. It is also heavily dependent upon immunological competency and thus strongly influenced by receipt of antiretroviral therapy. The risk of tuberculosis is increased 16-fold in HIV-positive individuals with a CD4 cell count below 200 but only 1.7-fold among people with HIV on antiretroviral therapy (11). In many countries where HIV is endemic, more than 50% of new cases of tuberculosis occur in HIV positives, and this figure reaches 70% in some regions of sub-Saharan Africa (12). Although the high risk of tuberculosis in people with HIV can result either from increased risk of reactivation disease or from unmasking of subclinical tuberculosis by antiretroviral therapy (13), most active cases of tuberculosis among patients with HIV infection result from new infection with *M. tuberculosis* (14–16).
This implies that diminished immune function in HIV infection impairs the relative protection against tuberculosis reinfection that has been observed in healthy subjects (17). The net effect of increased rates of both reactivation and new infection is that in areas of the world where tuberculosis is endemic, it is the most common cause of death in persons with HIV infection (12).

Isoniazid preventive therapy provides potent, if not durable, protection from reactivation tuberculosis among HIV-infected individuals (18–20), and antiretroviral therapy reduces the risk of tuberculosis by 80% (21, 22). With the decreased sensitivity of the tuberculin skin test and interferon gamma (IFN-γ) release assays (IGRAs) among HIV-infected individuals and the substantial challenge of delivering antiretrovirals in developing countries, improved immunization strategies against tuberculosis are a desperate need for persons with HIV infection.

**Tuberculosis in Other Immunocompromised Hosts**

Heightened risk of tuberculosis is described for multiple immunodeficiency states besides HIV infection. Heritable defects of IFN-γ and interleukin 12 signaling are associated with dramatic susceptibility to mycobacterial disease (23), as are multiple acquired immunodeficiency states, such as receipt of tumor necrosis factor alpha antagonists and other immunosuppressive therapies (24–26). Malnutrition has long been recognized to contribute to tuberculosis endemicity in the developing world (27, 28), presumably through an effect on immune function. Obesity has been noted to protect against tuberculosis among persons with HIV infection (29), yet the immunologic impairment associated with diabetes is increasingly recognized as an important and increasingly prevalent risk factor for tuberculosis (30). The safety and efficacy of any new tuberculosis vaccine will need to be tested in a wide variety of immunodeficiency states.

**Tuberculosis in Health Care Providers and Other International Travelers**

Health care providers, international outreach workers, and others working in areas where tuberculosis is endemic have a heightened risk for developing tuberculosis compared to that of the general population in low-prevalence countries (31, 32). Tuberculosis transmission to health care providers and other international travelers poses a health threat to individual travelers and could fuel the global spread of multidrug-resistant (MDR) and extensively drug-resistant tuberculosis. As a result, targeted BCG immunization for at-risk travelers to areas where MDR tuberculosis is endemic is being considered for reintroduction into national immunization guidelines in the United States.

**Sources of Immune Protection Against Tuberculosis**

At least partial immune protection from tuberculosis disease results from prior mycobacterial infection, whether naturally acquired or vaccine induced (Table 1). This observation implies the generation of immune responses to shared mycobacterial antigens, although it is possible that exposure to NTM exerts a nonspecific impact on the immune response to tuberculosis infection. Regardless, the magnitude of this protection is often not quantified and unlikely absolute.

**Natural Infection or Disease Due to M. tuberculosis**

Epidemiological and experimental animal studies indicate that prior infection with the tuberculosis agent confers relative protection against subsequent disease due to reexposure (33, 34). Such protection seems to be diminished in the face of cellular immunodeficiency, exemplified by reports of reinfection with new strains of M. tuberculosis among patients with untreated HIV infection (14). Recent data from South Africa indicated that both HIV-positive and HIV-negative persons with a first episode of active tuberculosis exhibit substantially increased risk of a second episode of active tuberculosis (35, 36). This raises the possibility that there are as-yet-unidentified host genetic factors which affect susceptibility to tuberculosis. The fact that reinfection has now been demonstrated in some persons does not contradict the prevailing view that most healthy persons with tuberculosis have some level of protection against reinfection. Nor does it suggest that protective immunization against tuberculosis is not possible. Population-based studies will be required to assess the magnitude of the protective effect of prior infection and its implications for tuberculosis vaccine development.

**Table 1** Known sources of protection against tuberculosis in humans

<table>
<thead>
<tr>
<th>Naturally-acquired mycobacterial infection</th>
<th>M. tuberculosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTM</td>
<td></td>
</tr>
<tr>
<td>Vaccine-induced mycobacterial infection</td>
<td>BCG (live)</td>
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<tr>
<td></td>
<td>M. microti (live)</td>
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<tr>
<td>Whole-cell mycobacterial vaccines (inactivated), including SRL 172</td>
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Natural Infection with NTM

Skin test studies with humans suggest that prior infection with NTM, acquired naturally from exposure to colonized water or soil, confers protection against tuberculosis (37, 38). Experimental data for animals demonstrate that infection with NTM protects against tuberculosis (38, 39). Infection with NTM is common in most regions of the world (40), and infections are usually acquired in childhood (41). In the United States, approximately 40% of adults have positive skin test reactions to NTM of the Mycobacterium avium complex (MAC), and most of these adults have negative tuberculin skin tests (42). Naturally acquired NTM infection may produce levels of protection against tuberculosis equal to that of BCG, and high rates of background infection with NTM in older children and adults have been proposed as an explanation for the lack of efficacy of BCG in some areas of the world (38). Prior mycobacterial infection may also reduce the efficacy of BCG by limiting its replication. Recent data indicate that infection of mice with environmental mycobacteria inhibits BCG replication and induction of a BCG-mediated immune response and impairs the protective effect of BCG after challenge with M. tuberculosis (43).

Immunization with Live Mycobacterium microti

The Medical Research Council conducted a controlled clinical trial of another live mycobacterial vaccine, Mycobacterium microti (the vole bacillus), in 1950. A single dose of M. microti was found to have a 5-year efficacy rate of 84%, equivalent to that of BCG, in a trial involving 54,239 tuberculin-negative British adolescents (44). These data indicate that antigens other than those derived from M. tuberculosis or M. bovis may protect humans against tuberculosis. Immunologic techniques were not available to assess mycobacterium-specific cellular immune responses; thus, the in vitro correlates of protection with this vaccine have not been identified.

Immunization with Inactivated Whole-Cell Mycobacterial Vaccines

Inactivated whole-cell mycobacterial vaccines were tested before the widespread acceptance of BCG and were shown to be effective in preventing tuberculosis in humans. Multiple doses were required, and animal studies indicated that the inoculum had to be higher than for BCG since replication did not occur after immunization (45). Jules Freund demonstrated that a multidose series of heat-killed M. tuberculosis had an efficacy of 42% against tuberculosis in a controlled clinical trial in the 1930s (46). More than 100,000 Italian children have been immunized with inactivated whole-cell mycobacterial vaccines (including a vaccine that combined M. tuberculosis, M. bovis, and M. avium), and a study with more than 18,000 of these children showed a reduction in the tuberculosis mortality rate from 5% in unimmunized children to 0% in immunized children (45).

In 2010, our group showed in a 7-year phase III randomized clinical trial with 2,013 HIV-infected adults in Dar es Salaam, Tanzania, with prior BCG immunization and CD4 cell counts over 200 cells/μl that immunization with a heat-inactivated whole-cell mycobacterial vaccine, SRL 172, resulted in a 39% reduction in culture-confirmed tuberculosis (47). This represents the first proof of efficacy of a BCG booster vaccine, and the first new tuberculosis vaccine to show efficacy specifically in adults with HIV infection.

MECHANISMS OF IMMUNE CONTROL OF TUBERCULOSIS

Macrophage and T-Cell Containment of Tuberculosis

Cellular immunity is essential to protection from tuberculosis. After initial infection and systemic dissemination, M. tuberculosis engages with Toll-like receptors 2 and 4 as well as other surface receptors on macrophages, and phagocytosis ensues. The resultant local and systemic cytokine cascade promotes T-cell recruitment to sites of tissue infection. Toll-like receptor activation and signaling by IFN-γ and other Th1 cytokines from T cells and nearby macrophages foster M. tuberculosis auto-phagy (48–50) as well as the degradation of M. tuberculosis by reactive oxygen and nitrogen intermediates within the maturing phagosome (51, 52).

The promotion of intracellular degradation of M. tuberculosis within macrophages by Th1 cytokines secreted by CD4+ T cells is considered the most important component of long-term immune defense against tuberculosis. This observation is based on the observation that major histocompatibility complex (MHC) class I knockout mice are less susceptible to tuberculosis than MHC class II knockouts (53). However, this model has been complicated by the identification of a number of CD4+ T-cell subsets that participate in the immune response to M. tuberculosis, including memory T-cell subsets and γδ T cells, as well as regulatory and Th17 T cells (54–57), with the relative contribution of each to immune protection from tuberculosis awaiting eluci-
dation. Furthermore, the contribution of CD8+ T cells to protection against tuberculosis is being reconsidered in light of the identification of αβ T-cell receptor-positive, CD4+, non-MHC-class I-restricted T-cell responses to tuberculosis (56) and data from a nonhuman primate model in which susceptibility to tuberculosis was dramatically enhanced after antibody depletion of CD8+ T cells (58). A major study with 5,662 BCG-immunized neonates in South Africa showed no correlation between any CD4+ and CD8+ T-cell cytokine response to mycobacterial antigens and later protection from tuberculosis (59), suggesting that these responses do not define or contribute indispensably to vaccine-mediated protection from tuberculosis among most BCG recipients.

Beyond the clear importance of cellular immune responses to immune protection from tuberculosis, tuberculosis-specific antibodies have conferred protection from tuberculosis or reduced CFUs in lungs after tuberculosis challenge in mouse models, either via direct antibody-tuberculosis interactions or via antibody-mediated immune modulation (60). However, in a rhesus macaque model, B-cell depletion did not alter disease progression or clinical outcome after tuberculosis exposure (61), suggesting that antibody responses do not play a strong role in immune protection from tuberculosis.

**Intracellular Persistence of Tuberculosis**

The potent and multifaceted macrophage and T-cell immune response to infection with *M. tuberculosis* in healthy human hosts leads to successful containment of tuberculosis as latent disease in 90% of subjects. However, this immune response ultimately fails to eradicate *M. tuberculosis* in 10% of subjects and results in progression to active disease or later reactivation disease. Beyond host immunodeficiency, mechanisms for ongoing *M. tuberculosis* persistence in macrophages include inhibition of phagosomal maturation and lysosomal fusion and perturbations in calcium and iron homeostasis as well as cellular lipid metabolism (62–65). The result is an immunologic stalemate in which the coordinated responses of macrophages and T cells foster immune containment of *M. tuberculosis*, but instead of full eradication, long-term intracellular persistence of *M. tuberculosis* results, allowing for later reactivation in the setting of waning cellular immunity.

**Immunopathogenesis**

Inadequate immune responses to mycobacterial antigens inarguably contribute to host vulnerability to development of tuberculosis. More is not always better, however, in the immune response to tuberculosis. Higher bacillary loads and worse disease progression have been associated with the elicitation of type 1 IFN responses to mycobacterial antigens (66–68), and IFN-γ responses to mycobacterial antigens may contribute to HIV-related immune reconstitution inflammation syndrome (69–71) as well as the pathogenesis of active tuberculosis (72). Intensification of antmycobacterial immune responses by a tuberculosis vaccine thus could elicit protective or pathogenic immunity depending on its immunologic impact, a reminder of the critical importance of identifying the immunologic correlates of vaccine-mediated protection from tuberculosis.

**Antigen Specificity of the Protective Immune Response to Tuberculosis**

Amidst an increasingly complicated model of the protective immune response to tuberculosis, our understanding of the antigen specificity of the protective immune response against tuberculosis has evolved as well. Conventional CD4+ T cells clearly target immunodominant antigens such as early secretory antigenic target 6 (ESAT-6) and antigen 85 (Ag85), and data from a guinea pig model and observational studies with humans suggest greater protection from tuberculosis with immune responses targeting multiple rather than single antigens (73, 74). Furthermore, it is clear that the range of mycobacterial antigens targeted by CD4+ and CD8+ T cells is broad; it includes DosR regulon-encoded proteins (75) and antigens presented to non-classically restricted T cells (76–78).

The antigen specificity of the immune response to *Mycobacterium tuberculosis* has been defined primarily through examination of immunodominant cellular immune responses. The assumption behind this analysis approach is that the most frequently detectable and greatest-magnitude immune responses to mycobacterial antigens are those that are most likely associated with immune protection from disease. This hypothesis remains unproven. Further, the immune response to *Mycobacterium tuberculosis* includes cell types such as CD1-restricted T and NK T cells responding to lipid antigens (79), while γδ T cells target small phosphate-containing nonpeptidic antigen (80, 81), with the comparative importance of these responses to immune protection from tuberculosis also still unknown.

The increasing complexity of the working model of protective immunity to tuberculosis has had a practical consequence: it is unclear when assessing candidate vaccine immunogenicity which immune responses against which antigens will best correlate with the induction of a protective immune response against tuberculosis.
A pivotal challenge in this work is to determine whether the correlates of natural immune protection from tuberculosis are the same as the correlates of vaccine-mediated protection from tuberculosis or if in cases the two are distinct.

**BCG VACCINE**

**History**

BCG was developed by Leon Calmette and Camille Guérin, the former a physician, the latter a veterinarian. Beginning in 1902, they passaged a strain of *M. bovis* isolated from a cow with tuberculous mastitis in culture every 3 weeks for a total of 230 passages. Beginning in 1913, they inoculated calves and then guinea pigs with the attenuated *M. bovis* strain, with no evidence of infection. They subsequently challenged immunized cows with a wild-type, virulent strain of *M. bovis*, without any resulting evidence of infection. Protection was then demonstrated in pigs, rabbits, and horses. In 1921, the vaccine was first administered orally to humans. The first recipient was a 3-day-old infant whose mother had died of tuberculosis a few hours after giving birth. The grandmother also had advanced tuberculosis and was the child’s guardian. In these circumstances, which mimic the current recommendation for use of BCG in the United States by the American Academy of Pediatrics, the infant’s risk of developing infection with disseminated disease and/or meningitis was deemed to outweigh the unknown risks of the new vaccine. The child had no ill effects from the vaccine and was raised by the grandmother without developing tuberculosis. Between 1921 and 1924, the vaccine was given to an additional 600 children, without serious complications. Production and vaccination efforts were increased so that approxi-...
Tuberculin skin test responses
Most tuberculin-negative subjects who are immunized with BCG develop a positive tuberculin skin test several weeks later. This effect wanes with time, and thus, it is recommended that tuberculin reactions of >10 mm several years after immunization be interpreted as latent infection with *M. tuberculosis* rather than the persistent effect of BCG. However, BCG-induced tuberculin reactions are often larger than 10 mm and can be boosted with repeated tuberculin skin tests. Although it is generally stated that the development of BCG-induced tuberculin sensitivity is not a surrogate for protective immunity against tuberculosis, a volunteer study in the United States showed a correlation between BCG-induced tuberculin sensitivity with *in vitro* markers of immune response to BCG (69), and skin test responses to BCG challenge continue to be evaluated more definitively (101).

Antimycobacterial Functionality in Response to Immunization against Tuberculosis
Given the uncertain relationship between known measurable immune responses and vaccine-mediated protection from tuberculosis, new assays are in development to assess both BCG and new candidate vaccines. Of particular interest recently have been functional assays that assess not immune responses to mycobacterial antigens but, instead, vaccine-mediated suppression of mycobacterial growth. One example is the mycobacterial growth inhibition assay, which showed responses after primary but not booster BCG immunization, consistent with the known efficacy of primary but not booster immunization (101). Additional models in development include attenuated *Mycobacterium tuberculosis* for administration to humans after vaccination with a new candidate vaccine (102), preclinical candidate vaccine testing in a primate aerosol challenge model (103), and postvaccination challenge with intradermal BCG followed by skin biopsy assessment of BCG growth at the site of injection (104). As with cellular immune assays in vogue in recent years, it will be critical to remain dispassionate about whether functional assays truly predict vaccine-mediated protection from tuberculosis in preclinical evaluations.

Efficacy of BCG against Tuberculosis

**Overview**

Although most countries and international bodies have concluded that childhood BCG immunization is effective in the prevention of tuberculosis, this view was never widely accepted in the United States. The controversy is based largely on the variable results of BCG efficacy trials and on different interpretations of these trials. Reexamination of the major prospective trials in light of contemporary knowledge of mycobacterial immunity and an improved understanding of critical trial design issues supports the view that childhood immunization is effective. Retrospective analysis of tuberculosis risk in subjects with and without BCG scars is subject to potential confounding by socioeconomic status (i.e., those without childhood BCG immunization may have come from lower socioeconomic groups and be at an inherently higher risk of tuberculosis) and is not useful in assessing the efficacy of BCG.

Because prior infection with either *M. tuberculosis* or NTM confers protection against tuberculosis comparable to that induced by BCG, vaccine efficacy for childhood immunization programs can be assessed adequately only by prospective trials of BCG immunization in mycobacterium-naive hosts, i.e., newborns. Numerous older trials attempted to screen out older children and adults with preexisting mycobacterial immunity by using intradermal skin tests. However, contemporary *in vitro* studies demonstrate that many skin test-negative subjects have demonstrable cellular and humoral immune responses to mycobacteria and are therefore not mycobacterium naive (90, 95, 105–107). Accordingly, retrospective studies that compare rates of tuberculosis in subjects with and without BCG scars are subject to potential bias, since the absence of immunization might reasonably be expected to correlate with lower socioeconomic status, which is itself a risk factor for disease (108). Thus, efficacy trials should be separated into those conducted with mycobacterium-naive newborns and those conducted with mycobacterium-experienced older children and adults using newer assays of exposure to mycobacteria.

**Trials in mycobacterium-naive subjects**

Four prospective trials have assessed the efficacy of BCG immunization against tuberculosis in newborns and infants (*Table 2*) (85, 109–112). Collectively, these trials demonstrate an efficacy of 73% against disease and 87% against death. An exemplary trial in this group was the randomized, placebo-controlled study conducted in Chicago, IL, in the 1930s by Rosenthal et al. (111). Participants were infants less than 3 months of age, and BCG was given by the multiple-puncture technique. Approximately 1,700 subjects were enrolled in each arm and monitored for 12 to 23 years; a vaccine efficacy of 74% was demonstrated. Trials with children have demonstrated that BCG is 86% effective in the prevention of
TABLE 2  Efficacy of BCG against tuberculosis: trials in newborns

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Yr</th>
<th>Location</th>
<th>Subjects</th>
<th>No.</th>
<th>% efficacy (disease)</th>
<th>% efficacy (death)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Aronson</td>
<td>1948</td>
<td>Western United States</td>
<td>Newborns (Native Americans)</td>
<td>232</td>
<td>59</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>Ferguson and Simes</td>
<td>1949</td>
<td>Montreal, Canada</td>
<td>Newborns</td>
<td>609</td>
<td>80</td>
<td>78</td>
<td>109</td>
</tr>
<tr>
<td>Rosenthal et al.</td>
<td>1960</td>
<td>Chicago, IL</td>
<td>Newborns</td>
<td>451</td>
<td>74</td>
<td>100</td>
<td>110</td>
</tr>
<tr>
<td>Rosenthal et al.</td>
<td>1961</td>
<td>Chicago, IL</td>
<td>Newborns</td>
<td>3,381</td>
<td>72</td>
<td>84</td>
<td>111</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>4,673</td>
<td>73</td>
<td>87</td>
<td></td>
</tr>
</tbody>
</table>

bacteremic disease, including disseminated tuberculosis and tuberculous meningitis (113).

Trials with mycobacterium-experienced subjects

Numerous BCG trials have been conducted with older children and adults. Each of these trials is subject to potential bias introduced by including subjects who have already been infected with M. tuberculosis (in regions where tuberculosis is endemic) or NTM (in most areas of the world). As noted above, contemporary in vitro immunologic techniques suggest that the tuberculin skin tests used to screen out mycobacterium-experienced subjects may not have been sufficiently sensitive to identify preexisting mycobacterial immunity. Thus, negative trials in this category may simply have been an attempt to immunize persons with naturally acquired immunity.

However, some studies with older children and adults demonstrated the protective efficacy of BCG immunization. One example is the large trial conducted between 1935 and 1938 with Native Americans by Aronson (85). This was a randomized, placebo-controlled study of persons aged 0 to 20 years (28% less than age 5) with baseline single and extra-strength tuberculin screening to exclude those with prior mycobacterial exposure. The original study enrolled 3,287 participants. Evaluation at 11 years demonstrated a 75% reduction in radiographically diagnosed tuberculosis, and evaluation at 20 years demonstrated an 82% reduction in mortality. Overall vaccine efficacy was 70%. A recent report provided the longest-term follow-up of any BCG trial: data on 1,998 of 2,963 original participants showed a vaccine efficacy of 52% against disease after 50 to 60 years (114).

Another well-designed prospective study conducted among 14- to 15-year-old British schoolchildren in the 1950s enrolled over 25,000 subjects in the vaccine and control groups. Baseline screening excluded children with reactions to standard or extra-strength tuberculin. Vaccine efficacy was determined to be 76% over a follow-up period of 15 years (44).

Chingleput, South India, trial

The South India trial deserves special consideration since it was designed as the ultimate randomized, controlled trial to investigate the protective efficacy of BCG against tuberculosis (115, 116) and is often cited to show that BCG is not effective. The study had the objectives of comparing the efficacies of different BCG strains and doses and assessing the efficacy of BCG in those with and without prior infection (determined by baseline tuberculin skin testing). In fact, the trial was principally a study of the effect of BCG in older children and adults, many of whom were already tuberculin positive and all of whom lived in an area of high leprosy prevalence. The trial was initiated in 1968 and enrolled over 270,000 subjects, but it enrolled only 1,500 (0.6%) aged 0 to 1 month for randomization to vaccine or placebo. Surveillance for tuberculosis was based on a positive chest X ray, and these were performed only at age 5 or above; those with positive X rays had sputum microbiology. Tuberculosis endpoints were said to be positive only if the subject had a positive sputum culture or positive acid-fast bacillus stain, and there were no methods for detecting extrapulmonary tuberculosis. Collectively, these endpoint definitions would be very insensitive for detecting tuberculosis in children. Further, surveillance in the overall study was not uniform for all subject groups, and the rate of tuberculosis endpoints was only half the predicted rate. Lastly, when the multivariate analyses were restricted to the subset of 40,342 study subjects without baseline skin test reactions to NTM, BCG immunization was associated with a 32% reduction in the risk of tuberculosis (117). The most reasonable interpretation of this large trial is that BCG vaccination of mycobacterium-experienced older children and adults in India did not lead to reduction in sputum culture-positive pulmonary tuberculosis.

Trials in HIV infection

In spite of the substantial burden of tuberculosis-related morbidity and mortality among people infected with HIV, and the potential for HIV infection to compromise
immune responses to BCG vaccination, there are no prospective trials of the efficacy of BCG immunization in the prevention of tuberculosis in persons with HIV infection. Retrospective and case-control studies have provided conflicting results, with one study suggesting protection against disseminated tuberculosis (118) and another showing no protection (119). Since BCG immunization in HIV-infected infants carries a risk of disseminated BCG disease, vaccine should not be administered to infants with known HIV infection (118–122).

Interpretation of trials
Two meta-analyses have evaluated the major prospective trials of BCG efficacy. A review by Clemens and colleagues evaluated the methodologies of 8 community trials with specific reference to susceptibility bias, surveillance bias, and diagnostic testing bias; confidence intervals for reported efficacy were also calculated. The three trials judged to meet strict methodologic criteria were the North American Indian, Chicago, and British trials: all showed protective efficacy (Table 3) (123). These 3 were also the only 3 with narrow confidence intervals; the remaining 5, including those that purported to show negative efficacy, had broad confidence intervals including negative and positive efficacies. The South India trial was not interpreted to have adequate protection against surveillance bias and diagnostic testing bias.

A review by Colditz and colleagues analyzed 14 prospective trials and 12 case-control studies, including several that were considered to have inadequate methods by the analysis by Clemens et al. (124, 125). Based on both prospective and case-control studies, the Colditz reviewers concluded that the overall protective efficacies of BCG were 50% for disease, 71% for tuberculosis mortality, and 64% for tuberculous meningitis.

Vaccine trial endpoints: prevention of disease
Tuberculosis endpoints vary considerably in various tuberculosis vaccine trials, ranging from culture confirmation to clinical criteria. Two prospective BCG trials and one trial of a new TB vaccine used both definite (culture confirmed) and probable (clinical criteria) definitions of tuberculosis. All three showed vaccine efficacy against definite tuberculosis but either lower or absent efficacy against probable tuberculosis (114, 126, 127). This finding has three leading explanations: (i) misclassification of probable cases (which is unlikely when rigorous diagnostic criteria are used [47]); (ii) existence of two forms of tuberculosis disease, one of which is more amenable to vaccine-based protection; and/or (iii) a shift in diagnostic categories via vaccine-mediated reductions in bacillary load such that cases of culture-confirmed tuberculosis become cases of probable tuberculosis and those who would have had (more difficult to diagnose) probable diagnosis transition into a state of latency.

Vaccine trial endpoints: prevention of disease
Most studies have evaluated the efficacy of BCG for protection against active tuberculosis. Latent infection had not been assessed as an efficacy endpoint when the presence of latent infection could be assessed only by tuberculin skin test (whose utility is limited since BCG itself can sometimes induce a positive tuberculin test). However, since IGRA s are not affected by BCG, they can be used to assess M. tuberculosis infection risk after immunization. Multiple studies have now suggested that BCG may reduce the risk of M. tuberculosis infection after high-risk exposure (128). In a Turkish study, 979 children with exposure to pulmonary tuberculosis were evaluated for latent M. tuberculosis infection using a T-cell-based enzyme-linked immunospot assay (129). BCG vaccination was protective for latent infection (odds ratio, 0.60; 95% confidence interval, 0.43 to 0.83). A British study found a similar reduction in risk of M. tuberculosis infection after a high-risk school exposure (130). IGRA s can be used to test for protection against tuberculosis in recipients of new vaccines that do not contain M. tuberculosis antigens used in the IGRA s, but IGRA s cannot assess protection against infection after vaccination with vaccine candidates that include antigens with significant homology to the antigens used.

### Table 3
Efficacy of BCG against tuberculosis: trials meeting strict methodologic criteria

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Yr</th>
<th>Location</th>
<th>Subjects</th>
<th>No.</th>
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<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stein and Aronson</td>
<td>1953</td>
<td>Western United States</td>
<td>Age 0–20 yr (Native Americans)</td>
<td>3,008</td>
<td>67</td>
<td>82</td>
<td>204</td>
</tr>
<tr>
<td>Hart and Sutherland</td>
<td>1977</td>
<td>England</td>
<td>Newborns</td>
<td>26,465</td>
<td>76</td>
<td>NA</td>
<td>44</td>
</tr>
<tr>
<td>Rosenthal et al.</td>
<td>1961</td>
<td>Chicago</td>
<td>Newborns</td>
<td>3,381</td>
<td>72</td>
<td>84</td>
<td>111</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>32,854</td>
<td>71</td>
<td>82</td>
<td></td>
</tr>
</tbody>
</table>

*Studies selected based on analysis by Clemens et al. [123]. NA, not applicable.*
in IGRAs (131). Further, since impaired immunity can reduce sensitivity of IGRAs at the same time it heightens susceptibility to disease and may modify likelihood or potency of vaccine elicitation of immunity, IGRAs may misestimate vaccine efficacy.

**Duration of BCG benefit**

The duration of benefit of BCG immunization is controversial. There is strong evidence that BCG confers protection from tuberculosis for at least 10 years (132, 133), and additional studies suggest more durable protection at least in some populations. BCG conferred protection from tuberculosis for more than 50 years in American Indians and Alaska Natives (114), and neonates immunized with BCG in Brazil exhibited 15 to 20 years of protection (108). A recent study in Norway suggested that the majority of BCG vaccinees exhibited at least 10 to 19 years of protection from pulmonary tuberculosis (134).

**Variations in BCG Efficacy**

Other possible explanations for observed variations in the efficacy of BCG have included differences in potency of various strains, genetic or age differences in target populations, variations in efficacy against different forms of disease, and reduced virulence of some strains of *M. tuberculosis* (124). None of these hypotheses explain the observed variations as well as methodologic differences in the trials and/or differences in preexisting mycobacterial immunity.

**BCG revaccination**

Although a few countries still administer booster doses of BCG to tuberculin-negative children, there is no evidence that single-dose revaccination confers additional protection against tuberculosis (135–138), despite the enhancement of IFN-γ responses to mycobacterial antigens (139). There are two possible explanations for this finding. One is that since BCG must replicate to induce immunity (43), it may be that the newborn dose of BCG or subsequent infection with *M. tuberculosis* or NTM confers a sufficient immune response against the organism to prevent replication of the subsequent dose. The other is that older children and adults who are administered a booster may have already acquired additional immune protection against tuberculosis from infection with *M. tuberculosis* or NTM.

**Efficacy of BCG against Other Diseases**

**Mycobacterium leprae**

Several studies have demonstrated an efficacy of 50 to 80% against *M. leprae*, and this effect may be increased with booster doses of BCG (138), although these data are inconsistent (137).

**Mycobacterium ulcerans**

BCG has been shown to be approximately 50% effective in preventing Buruli ulcer disease due to *M. ulcerans* (140). This includes protection against osteomyelitis, a major complication of *M. ulcerans* infection (141). A more recent case-control study failed to detect a protective effect (142).

**MAC**

BCG also provides cross protection against childhood lymphadenitis due to MAC (143). Cessation of childhood BCG immunization has been associated with a marked increase in the rate of childhood adenitis due to NTM (6).

**Childhood mortality**

In addition, BCG immunization of children in developing countries has been associated with reduced all-cause mortality (144, 145). This effect is not specifically attributable to reduction in mortality from tuberculosis and is not fully understood.

**Administration of BCG**

Aventis Pasteur has withdrawn its license to distribute BCG in the United States for the prevention of tuberculosis, leaving the reconstituted Tice vaccine (Organon) as the sole licensed tuberculosis vaccine in the United States. The Tice vaccine contains a mixture of killed and live bacilli with a range of 37,500 to 3,000,000 CFU per dose (146), and the manufacturer recommends that 0.2 to 0.3 ml of vaccine reconstituted in 1.0 ml of sterile water be administered in the lower deltoid area by the multiple-puncture technique (0.2 to 0.3 ml reconstituted in 2 ml of sterile water for infants less than 1 year of age). Individual manufacturers’ instructions should be consulted. Reconstituted vaccine should be refrigerated and should be protected from exposure to light. Unused vaccine should be discarded after 2 to 4 h and should be treated as infectious waste, as should all equipment used in vaccine preparation and manufacture. Tuberculin skin test conversion usually occurs 6 to 12 weeks after immunization.

**Side Effects of BCG**

**General**

Side effects of BCG immunization have been shown to be dependent on the BCG strain, dose, method of administration, and recipient (147). Neonates are more...
likely to experience complications than older children and adults. Small clusters of increased complication rates have been associated with a change in the strain or method of vaccination. Among strains currently in use, the Pasteur and Danish have been associated with the highest rate of side effects. For example, lymphadenitis is more common with the Pasteur strain than with the Tokyo or Brazil strain (148). The average CFU of viable bacilli varies by vaccine strain, and most products also include nonviable bacilli. Intradermal inoculation is associated with a higher rate of local reactions. The multiple-puncture technique has a lower rate of local reactions but is more costly, is less precise, is time-consuming, and is technically involved (148). Adverse effects of BCG immunization are summarized in Table 4.

Common and local reactions
The most common side effect of BCG is a local reaction at the site of inoculation characterized by pain, swelling, and erythema. This is seen in 95% of vaccine recipients, typically lasts several weeks, and usually resolves by 3 months without any complication other than scar formation (149). Approximately 75% of vaccinees also experience some myalgia. Seventy percent have ulceration with drainage at the vaccine site. Vaccine site abscess has been reported for 2% of recipients and regional lymphadenitis for 1 to 2% (150, 151).

Among those who develop adenitis, ulceration with drainage is more likely if the lesion develops rapidly and within 2 months after vaccination. Surgery is usually required if fistulas and drainage develop. The role of adjunctive antimycobacterial therapy remains controversial. More indolent and later developing lesions are best managed with observation alone (152, 153).

Osteomyelitis
Osteomyelitis has been reported at a rate of between 0.01 per million vaccinees in Japan (multipuncture technique) and 300 per million in Finland (intradermal technique) (151). Treatment of osteomyelitis is with isoniazid and rifampin (BCG is resistant to pyrazinamide).

Disseminated disease
Disseminated disease, including fatal outcome, is reported at an overall rate between 0.19 and 1.56 per million vaccinees. Prior to the HIV epidemic, most cases of disseminated BCG occurred in infants with unrecognized severe combined immunodeficiency (SCID). Studies conducted in South Africa have documented disseminated BCG rates in the range of 407 to 1,300/100,000 HIV-infected infants (154). However, studies in other regions and with different BCG strains have found lower risk or no risk (155). Treatment is with isoniazid and rifampin (156).

Current Use of BCG
Developing countries
BCG is administered routinely to newborns in countries where tuberculosis is endemic (157). Vaccine is typically administered over the deltoid or on the forearm. Because BCG is included in list of recommended childhood immunizations by the World Health Organization, current coverage is >80% in many countries. In countries with endemic HIV infection, the World Health Organization recommends testing infants for HIV infection prior to administration of BCG. This recommendation should not be interpreted to cease routine BCG immunization of infants if HIV testing cannot be implemented (154).

Developed countries
BCG is still administered widely at birth in developed countries such as Ireland and Monaco and selectively to high-risk infants in other developed countries such as the United Kingdom, where the incidence of tuberculosis has fallen (157–159). Selective immunization can strike a reasonable balance between protection and vaccine side effects in countries where the general incidence of tuberculosis is falling but high-risk groups can be identified at birth (160).

BCG has never been administered routinely in the United States but was used more widely before the in-

### Table 4: Adverse effects of parenteral BCG immunization

<table>
<thead>
<tr>
<th>Reaction type</th>
<th>Incidence</th>
<th>Comment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection site induration, pain, erythema</td>
<td>95%</td>
<td>Essentially all vaccines</td>
</tr>
<tr>
<td>Ulceration at inoculation site</td>
<td>70%</td>
<td>Varies with strain; increased in neonates</td>
</tr>
<tr>
<td>Local ulceration/adenopathy</td>
<td>1–2%</td>
<td></td>
</tr>
<tr>
<td>Serious</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>0.01–300/million</td>
<td>Varies with strain</td>
</tr>
<tr>
<td>Disseminated infection</td>
<td>0.19–1.56/million</td>
<td>Associated with immunocompromised state (CGD, SCID, HIV, etc.)</td>
</tr>
</tbody>
</table>

*Based on references 147 to 149, 150, and 205 to 207. CGD, chronic granulomatous disease; SCID, severe combined immunodeficiency.
cidence of tuberculosis reached its current low levels. For example, health care workers were often immunized in the last century, and many physicians and nurses who practiced in that era still have BCG scars (161). Because U.S. policy for the prevention of tuberculosis places a strong emphasis on tuberculin skin testing and treatment of latent infection, and because BCG may interfere with the tuberculin skin test, there has been a strong reluctance to endorse BCG for all potential high-risk groups. Current guidelines from the American Academy of Pediatrics and from the CDC Advisory Committee on Immunization Practices are listed in Table 5. The guidelines recommend BCG for a child who is continually exposed to a person with untreated or ineffectively treated tuberculosis and who cannot be given antituberculosis therapy. Additionally, BCG is recommended for a child exposed to a person with MDR tuberculosis when the child cannot be removed from contact with the index case. These guidelines are sufficiently restrictive that BCG manufacturers have been reluctant to distribute vaccine in the United States. The guidelines do not include other important other high-risk groups, such as homeless persons in the United States (162), medical relief personnel from low-incidence countries working in areas where tuberculosis is endemic (163), and U.S. children moving to countries where tuberculosis is endemic. A revision of BCG guidelines by the World Health Organization is currently under way (164) and will likely include a recommendation for immunization of health care and medical relief workers working in regions where they may be exposed to MDR tuberculosis (165). Until then, the predominant use of BCG in the United States is topical installation in the bladder for treatment of bladder cancer (166).

NEW VACCINES AGAINST TUBERCULOSIS

Rationale

The favorable and unfavorable characteristics of BCG are summarized in Table 6. The efficacy of BCG against tuberculosis is high when BCG is administered to newborns, but this protection from tuberculosis disease likely wanes into adulthood, and there is likely minimal, if any, protection against reactivation tuberculosis in adults. In addition, BCG boosters are ineffective (135). There is thus wide consensus that additional tuberculosis vaccine development should focus on identifying a booster vaccine to follow BCG immunization or on a completely novel two-vaccine booster regimen. Modeling studies indicate that an adolescent and adult booster would have a greater impact on the epidemic in the initial decades after introduction (167). Although enhanced potency is cited as one goal of new tuberculosis vaccine development, equally important goals include reducing the risk of side effects and improving the duration of protection. Because HIV-associated tuberculosis may now account for more than 50% of global cases of tuberculosis, another important goal is the development of a safe, effective, and durable vaccine strategy for the prevention of HIV-associated tuberculosis (168). Further, new vaccines should be economical, and single-dose vaccines would be preferable to those that require multiple doses. It would also be advantageous to have a vaccine that does not require parenteral immunization (169). Fortunately, the development of IGRAs has made vaccine-mediated interference with tuberculin skin test responses less critical for new tuberculosis vaccine candidates.

Animal Testing

Candidate vaccines are typically screened for immunogenicity and protective efficacy in the mouse model. BCG vaccine is used as a gold standard and generally produces a 0.7-log reduction in CFU in the lung after virulent M. tuberculosis challenge (170). However, disease in mice differs in several respects from disease in

### TABLE 5 Recommendations for BCG use in the United States

<table>
<thead>
<tr>
<th>Advisory group recommendations</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with continuous exposure to a person with contagious MDR tuberculosis (and who cannot be removed from contact with the person)</td>
<td></td>
</tr>
<tr>
<td>Children with continuous exposure to a person with untreated or ineffectively treated pulmonary tuberculosis (and who cannot be removed from contact with the person)</td>
<td></td>
</tr>
<tr>
<td>Health care workers exposed to contagious MDR tuberculosis in settings where infection control programs have failed to prevent transmission</td>
<td></td>
</tr>
<tr>
<td>Additional recommendations</td>
<td></td>
</tr>
<tr>
<td>Tuberculin-negative homeless persons</td>
<td>(162)</td>
</tr>
<tr>
<td>Infants or tuberculin-negative adults moving to countries where tuberculosis is endemic</td>
<td></td>
</tr>
<tr>
<td>Health care workers (medical students, physicians, nurses, etc.), medical relief workers, missionaries, and others traveling to conduct direct patient care activities in countries where tuberculosis is endemic</td>
<td>(163)</td>
</tr>
<tr>
<td>Contraindications</td>
<td></td>
</tr>
<tr>
<td>HIV infection or other immunodeficiency (e.g., SCID, DiGeorge syndrome)</td>
<td></td>
</tr>
<tr>
<td>Hematologic or generalized malignancy</td>
<td></td>
</tr>
<tr>
<td>Immunosuppression (e.g., TNF blocking agents, chronic steroid therapy, alkylating agents, antimitabolites, radiation)</td>
<td></td>
</tr>
<tr>
<td>Positive tuberculin skin test or prior tuberculosis</td>
<td></td>
</tr>
</tbody>
</table>

*SCID, severe combined immunodeficiency; TNF, tumor necrosis factor.
*See reference 208.
*See reference 209.
TABLE 6  Characteristics of BCG

<table>
<thead>
<tr>
<th>Favorable characteristics</th>
<th>Unfavorable characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn immunization reduces risk of disease and death due to childhood tuberculosis</td>
<td>Limited efficacy against reactivation disease</td>
</tr>
<tr>
<td>Newborn immunization reduces risk of miliary and meningeal tuberculosis</td>
<td>Limited efficacy in mycobacterium-experienced children and adults</td>
</tr>
<tr>
<td>Newborn immunization reduces risk of childhood nontuberculous lymphadenitis, leprosy, and M. ulcerans infection</td>
<td>Uncertain efficacy in HIV infection</td>
</tr>
<tr>
<td>Low cost</td>
<td>Limited duration of efficacy</td>
</tr>
<tr>
<td></td>
<td>Genetic variation in licensed vaccine strains</td>
</tr>
<tr>
<td></td>
<td>Risk of BCG adenitis or osteomyelitis in healthy recipients</td>
</tr>
<tr>
<td></td>
<td>Risk of disseminated BCG in HIV-infected recipients</td>
</tr>
<tr>
<td></td>
<td>Absence of booster effect</td>
</tr>
<tr>
<td></td>
<td>Requirement for parenteral immunization</td>
</tr>
<tr>
<td></td>
<td>Effect on skin test reaction to tuberculin</td>
</tr>
<tr>
<td></td>
<td>Unknown immune correlate of protection</td>
</tr>
</tbody>
</table>

Humans. For example, latent tuberculosis does not occur in mice, and thus, it is not known whether vaccines which reduce lung CFUs in mice will prevent tuberculosis in humans. There are examples where this model is not predictive. For example, *M. microti* is effective in humans but was found to have only marginal activity in the standard mouse model (170). Recent studies with the mouse model indicate that the immune response to challenge depends on the inoculum: higher inocula produce immune responses resembling immune responses in humans naturally exposed to tuberculosis, reinforcing that extrapolation from the animal model (171).

The guinea pig is more sensitive than the mouse to *M. tuberculosis* infection and demonstrates higher CFUs after challenge and progressive lung pathology. In this model, protection can be assessed using the endpoints of survival time and degree of lung pathology (170). Macaques and cynomolgus monkeys can also be used for preclinical evaluation of vaccine candidates and have several advantages over rodent models (disease more closely mimics human tuberculosis, antigen presentation and T-cell receptor repertoire are similar to those in humans, and the safety evaluation is more relevant), but testing in primates is expensive (172).

Despite innovative work to identify improved animal models (173, 174), animal model immunogenicity is not certain to correlate with vaccine-mediated protection against tuberculosis in humans and thus should not be the primary determinant of candidate vaccine advancement beyond preclinical trials (175).

**Human Trials**

Candidate vaccines are tested for safety first in small numbers of healthy adults and then in children and subsequently in immunocompromised subjects, such as those with HIV infection. Safety trials are conducted in both mycobacterium-naive and mycobacterium-experienced populations, including subjects with and without prior BCG and subjects from regions where tuberculosis is endemic. Safe vaccines then proceed to human immunogenicity (phase II) testing in the same populations. Relevant immune responses are identified above and include polyfunctional and memory responses to a wide range of mycobacterial antigens by CD4+ and CD8+ T cells, antibody responses, and assays of immune cell proliferation. Subsequent phase II studies are designed to determine optimal doses and schedules. Controlled efficacy trials (phase III) then follow. Trials with adult subjects can be targeted to high-risk subjects to reduce sample sizes and follow-up periods (176). Household contacts of tuberculosis patients and persons with early HIV infection would both be suitable. In pediatric and HIV-positive subjects, both pulmonary and bacteremic tuberculosis should be used as endpoints (177). Based on early successes in other fields (178), investigators and funders alike are warming to the idea of performing adaptive clinical trials that test multiple tuberculosis vaccine candidates simultaneously, with poorly performing candidates dropped at prespecified intervals (179). The efficiency and speed of such models are appealing, although challenges include the need for multiple funders and investigators to cooperate on identification of consensus endpoints.

**Vaccine Candidates**

Many new candidate tuberculosis vaccines are in various stages of development and testing (180). Selected vaccines with the most advanced human clinical studies are depicted in Fig. 1.

New vaccines in development aim either to replace BCG as the primary vaccine against tuberculosis or to boost preexisting BCG-mediated responses.

The two leading vaccines that aim to replace BCG are polyantigenic whole mycobacterial vaccines that theoretically should elicit at least as broad protective immunity but hopefully responses that are more potent and/or more durable. Live recombinant BCG (rBCG; also known as VPM1002) and live attenuated tuberculosis (MTBVAC) are both in phase II trials in 2016.

Most new vaccine development has focused on the identification of a booster vaccine for BCG. There are
three major types of new booster vaccines in development: whole-cell/polyantigenic vaccines, protein/adjuvant vaccines, and virally vectored vaccines.

Whole-cell/polyantigenic vaccines tested to boost BCG-mediated protection from tuberculosis include BCG itself and an inactivated whole-cell NTM, DAR-901. Their use is predicated partly on animal studies showing the efficacy of whole-cell inactivated vaccines and suggesting that antigens, including those derived from cell wall, membrane, and cytosolic components of the organism, may be important in mediating immune protection from tuberculosis (181). Further, based on data showing that polyantigenic IFN-γ responses to mycobacterial antigens were associated with greater natural immune protection from tuberculosis than IFN-γ responses against single antigens (74, 182), we hypothesize that polyantigenic vaccines will provide superior protection from tuberculosis compared to that of vaccines with narrower antigenic content. These findings, and the observation of tuberculosis protection after immunization with BCG or live M. microti, have driven the continued use of whole-cell vaccines, like BCG, and the development of novel whole-cell vaccines (183, 184).

Single-dose BCG boosters do not elicit additional protection from tuberculosis and have been abandoned (135–138). Recombinant versions of BCG have been engineered to enhance vaccine immunogenicity. Examples include recombinant BCG engineered to overexpress Ag85 or the rBCG Prague strain expressing listeriolysin, which improves antigen presentation by enhancing antigen escape from the phagosome (185). It remains to be seen if the enhanced immunogenicity of newer-generation BCG-based vaccines may come at a cost of exacerbated reactogenicity, and the efficacy of any live vaccine must be weighed against the risk of disseminated vaccine disease in immunocompromised hosts.

Our group showed that vaccination with an inactivated whole-cell NTM is safe and immunogenic, and in a phase III NIH-funded randomized placebo-controlled clinical trial in Tanzania, it had 39% efficacy in the prevention of microbiologically confirmed tuberculosis in BCG-immunized adults with HIV infection and CD4 counts of ≥200 cells/μl (47, 184, 186–188). Originally, the NTM in the vaccine studied by our group was designated M. vaccae, but subsequent genetic testing has confirmed its identity as M. obuense. The
original formulation of M. obuense was not amenable to worldwide scale-up, but we have now completed preclinical phase I testing to confirm safety and immunogenicity of a new formulation that will enter phase IIb trials in 2016.

Two types of booster vaccines now in development present antigens either in the context of a vaccine adjuvant or in a viral-vector platform, both of which elicit strong cytokine responses to candidate antigens. Candidate antigens are often selected for use in tuberculosis vaccines because they elicit detectable immune responses among the majority of individuals exposed to tuberculosis, perhaps via antigen or exposure on the surface of infected cells (3). Other antigens have been selected from those to which healthy PPD-positive donors (i.e., persons who have successfully contained latent infection) respond (189). Advantages of subunit vaccine approaches include safety, low reactogenicity, and easy evaluation of vaccine immunogenicity. Potential downsides include the elicitation of immune responses against a narrow antigen repertoire or among a narrowly defined subset of immune cells. In the largest and most advanced clinical trial of a virally vectored booster vaccine against tuberculosis, a phase IIb clinical trial of MVAg85A, the vaccine was safe and immunogenic in 2,797 BCG-immunized infants in South Africa but did not elicit protection from tuberculosis (190).

Most tuberculosis vaccines now in testing are delivered via subcutaneous or intramuscular injection. Yet mucosal immunization, either orally or via aerosolization, is still being investigated (191), in part because mucosal immunization with tuberculosis vaccine candidates elicits greater immune responses in mucosal sites such as the lungs (169, 192–195). There have been promising immunogenicity and aerosol challenge results in animal models of a lipid-encapsulated oral formulation of BCG (196–200), and in addition, an aerosolized version of MVAg85A is progressing through clinical trials. It will be important to determine if the efficacy of mucosal immunization against tuberculosis will be compromised by diarrheal illnesses prevalent in areas where tuberculosis is endemic, such as impacted the efficacy of oral polio vaccination programs (201).

CONCLUSIONS

BCG is associated with protection from tuberculosis in mycobacterium-naive hosts but is frequently associated with local side effects and also carries a risk of life-threatening disseminated BCG disease in newborns who have HIV infection. Despite global use of BCG immunization, morbidity and mortality from tuberculosis remain unacceptably high. Multiple new tuberculosis vaccine candidates are now in various stages of development, either to improve upon the safety and immunogenicity of BCG or to boost BCG responses.

ACKNOWLEDGMENT

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REFERENCES


American medical students, prevention and control and the use of BCG.


M. bovis BCG and New Vaccines


