Introduction

Tuberculosis (TB) claims approximately 2 million lives a year, close to 3 million if the estimate includes HIV+ individuals who die with active TB. There are 16.2 million total cases and 8 million new cases of active disease each year. Worldwide, the case fatality rate is 23%, with a greater than 50% rate reported from some African countries with high prevalence of HIV infection. One third of the earth's population (1.86 billion people) is believed 'latently' infected with *Mycobacterium tuberculosis* ([Figure 1](#)). A significant, although variable, proportion of active TB cases are due to reactivation of these 'latent' (or persistent) infections. Approximately 10% of immunocompetent individuals infected with *M. tuberculosis* will develop active TB at some time in their lives, and for those infected with HIV the risk of developing active TB is increased approximately 30-fold (to about 8% per year). The Global Burden of Disease Study determined that globally tuberculosis is the 7th leading cause of Disability Adjusted Life Years (DALYs), and unlike most infectious diseases, will still be among the top ten causes of DALYs in 2020.

BCG (live attenuated *Mycobacterium bovis* BCG) represents the only vaccine currently available against tuberculosis. It is the most widely administered of all vaccines in the WHO Expanded Programme for Immunization, but has been estimated to prevent only 5% of all potentially vaccine preventable deaths due to TB [The Children's Vaccine Initiative]. It has been shown to protect against disseminated and meningeal TB in young children, and to provide some
protection against leprosy, but its efficacy in preventing adult pulmonary TB, which carries the major burden of morbidity and mortality from this disease, has varied dramatically in carefully conducted studies throughout the world - from 77% in the UK to 0% in Chingleput, India. As a result of this variability in efficacy, the impact of BCG on the global TB epidemic has been negligible. BCG vaccine is not recommended for use in the US and some northern European countries because of its low efficacy and its interference with skin test screening. Current models predict that an effective TB vaccine would save tens of millions of lives over the next three decades, and that the beneficial effects of combining effective vaccination and treatment of active disease would be multiplicative.

History of TB Vaccine Development

BCG (bacille Calmette-Guerin) vaccines were developed by Calmette and Guerin in the early 1900s, by attenuation of virulent M. bovis via serial passage. BCG was first administered, orally, in 1921 to a newborn whose mother had died of TB and who was going to live with a grandmother suffering from the disease. This individual remained free of TB throughout his life. Between 1921 and 1927, 969 additional high risk children were vaccinated with BCG. Reportedly 3.9% died of TB or unspecified causes, while the mortality rate in a comparable group of unvaccinated children was 32.6%. As a result, the League of Nations in 1928 recommended widespread vaccination with BCG. The original BCG was not cloned, and distributed strains have been propagated throughout the world under varying conditions. This process created a variety of related "BCG" vaccines that today have varying genotypic and phenotypic characteristics. Molecular sequence and proteome comparisons are being performed to help determine to what extent, if any, the variability seen in BCG efficacy in different trials can be attributed to genetic differences. Such analyses should provide valuable clues to the genetic determinants of BCG vaccine efficacy.

In addition to genetic differences among BCG strains, a number of other potential causes of the observed variability in BCG efficacy have been proposed. These include: methodological differences among the clinical trials, genetic differences within and among host populations, varying levels of malnutrition among host populations, virulence differences among M. tuberculosis strains, varying efficacy of BCG against primary infection in children and endogenous reactivation of persistent infection versus exogenous re-infection, and effects of environmental mycobacteria on the host immune response to BCG. While it is unlikely that any one factor will fully explain the observed variability in BCG efficacy, among the listed possibilities, variable exposure to environmental mycobacteria may be the largest single contributor.

In addition to its variable efficacy and its interference with tuberculin skin test screening, BCG's usefulness is limited by its potential lack of safety in immunocompromised individuals: a number of cases of disseminated BCG
infection attributable to BCG vaccination have been reported in HIV+ and other immunocompromised individuals.\(^{13,14}\)

**Current State of the Science**

Tuberculosis research has seen a remarkable growth during the past decade. In 1989, the NIH spent $3M and supported a handful of grants on TB research, but this support increased almost 25-fold by 1999 to $73M and over 150 grants and contracts. A recent survey of major institutions' expenditures on TB research, conducted by the Global TB Programme of the WHO, estimated that worldwide expenditures in 1995 totaled $92M ($62M of which was from the NIH).\(^{15}\) This investment has led to significant scientific progress and has helped to develop a vigorous community of scientists devoted to elucidating the biology and pathogenesis of tuberculosis, and to developing improved tools, including effective vaccines, for control of this disease. Important advances include the complete genome sequencing of two strains of M. tuberculosis, a virulent laboratory strain, H37Rv,\(^ {16}\) and a recent, virulent clinical isolate CDC1551.\(^ {17}\) The M. leprae genome has recently been completed and genome sequencing of M. bovis, M. avium and M. smegmatis are underway, creating a rich mycobacterial sequence database and foundation for comparative functional analyses of these diverse pathogenic and non-pathogenic mycobacteria. The completed M. tuberculosis genome sequences also allow new antigen identification through implementation of microarray and proteomics analyses of gene products specifically expressed in vivo and during different stages of the disease process. A number of tools for efficient manipulation of mycobacterial genomes have also been developed,\(^ {18,19}\) allowing construction and analysis of specific mutants of interest and enabling further elucidation of gene function. Small animal models of primary pulmonary TB in the mouse, guinea pig and rabbit, as well as development of a cynomolgus monkey model, are enabling screening of large numbers of vaccine candidates for toxicity and efficacy in protecting against challenge with virulent M. tuberculosis (Fig. 2).\(^ {20,21,22}\) Importantly, models that mimic the persistent or "latent" phase of M. tuberculosis infection, exposure to environmental mycobacteria, and BCG vaccination are also being developed.

Relatively large numbers of potential vaccine candidates have been and continue to be developed and screened in these small animal models. To date, the NIAID TB Research Materials and Vaccine Testing Contract (PI = Dr. John Belisle, Colorado State University; N01 AI75320) has screened over 100 individual candidates in the mouse and/or guinea pig low dose aerosol challenge model. These fall into several broad categories:

- **Recombinant BCG vaccines** – expressing immunodominant antigens and/or cytokines.
- **Live, attenuated strains of M. tuberculosis** – including singly and doubly auxotrophic mutants.
- **Nonpathogenic mycobacteria** – including M. vaccae (a soil
mycobacterium, which has been tested as an adjunctive immunotherapeutic in adult pulmonary TB patients. M. microti (the vole bacillus; tested in humans by the British MRC), M. smegmatis (a rapidly-growing, non-pathogen) and M. habana (a slow-growing photochromogen originally isolated from monkeys).

- **Non-mycobacterial microbial vectors** – including attenuated Salmonella and Vaccinia virus, expressing immunodominant mycobacterial antigens.
- **Subunit vaccines** – protein-, lipid, and carbohydrate-based, but mostly protein/peptide antigens have been the focus to date.
- **DNA vaccines** - with various adjuvants; independently and in prime-BCG boost paradigms.

In addition, a whole genome screen of M. tuberculosis for protective antigens is being conducted by modified Expression Library Immunization, with initial screening in the mouse low dose aerosol challenge model. Protective pools have been deconvoluted and ten individual antigens are currently undergoing further testing in guinea pigs. A variety of recent findings suggest new avenues for exploration and provide additional fuel for optimism. These include, as examples:

- An expanding knowledge of T cell memory induction in vivo.
- An increasing understanding of innate immunity, including the discoveries that:
  - following mycobacterial lipid antigen presentation to T cells by CD1 proteins, toll-like receptors mediate cellular activation by *M. tuberculosis* and a protein in CD8+ CTLs, termed granulysin, directly kills *M. tuberculosis* in vitro.
- The demonstration that Hsp65 DNA vaccination effectively reduced bacterial load in mice with established virulent *M. tuberculosis* infection, and prevented 'reactivation' in a mouse model of chronic *M. tuberculosis* infection.

**Current Challenges**

Significant challenges to TB vaccine development remain, however. A recent study suggests in areas with high prevalence of tuberculosis, there may be higher than previously expected rates of exogenous re-infection in humans cured of primary tuberculosis. This result remains somewhat controversial, but an accumulating number of documented cases of re-infection confirm that complete natural protective immunity following cure of primary tuberculosis is not universal. Nonetheless, it must be remembered that 90% of all persons infected with *M. tuberculosis* mount a protective immune response that effectively provides life-long protection against the disease, and BCG
vaccination has been almost 80% efficacious in some settings in protecting against the development of tuberculosis. Thus effective vaccination is an achievable goal.

TB vaccine development would be greatly advanced by an improved understanding of the human protective immune response to *M. tuberculosis* infection, including the potential role(s) of various T cell populations and the molecular signals that activate the protective immune response. Adjuvant development will be an integral part of candidate development. Efficacy trials of candidate TB vaccines will likely be complex and expensive (see Table 1). These trials would be markedly simplified and potentially shortened by identification and validation of surrogate markers of protective immunity. The Tuberculosis Research Unit (TBRU; Principal Investigator: Dr. Henry Boom, Case Western Reserve University; N01 AI95383) and other groups are devoting significant effort to elucidating such markers. Low cost, simple diagnostics that could effectively distinguish *M. tuberculosis* infection from vaccination or exposure to environmental mycobacteria and from TB disease would be enormously useful both clinically and to facilitate clinical trials.

Additional research is also required to further define the antigens of *M. tuberculosis* that induce human protective immunity, develop improved animal models that better mimic the realities of human tuberculosis (including persistent infection and reactivation, and effects of nonpathogenic, environmental mycobacteria and BCG vaccination), and identification of the optimal route of human immunization.

The Power of Collaboration

The US National Institutes of Health and other funding agencies support much of the current basic and preclinical research in TB vaccine development, through investigator-initiated research grants and contracts such as the TBRU and TB Research Materials and Vaccine Testing effort. Infrastructure is made available to public and private sector partners for Phase 1 and 2 human trials (through the NIAID's TBRU and Vaccine and Treatment Evaluation Units, and CDC's TB Trials Consortium). Leadership has also recently been provided by the public sector in the development of a "Blueprint for TB Vaccine Development" and a DHHS Task Force convened by Asst. Secretary for Health and Surgeon General David Satcher to oversee further development and implementation of this plan. The US Food and Drug Administration is committed to assisting manufacturers in the development and licensure of new TB vaccines that are pure, potent, safe and effective. Recently private foundations (e.g., The Bill and Melinda Gates Foundation, the Wellcome Trust, the Sequella Global TB Foundation) have begun to make significant contributions to preclinical TB vaccine research and the establishment of an international clinical trial infrastructure. The World Health Organization (Steering Committee on Immunology of Mycobacteria, IMMYC) has served as an important forum for international and public-private sector discussions of needs in TB vaccine development, including spearheading discussions of potential efficacy trial designs, surrogate marker identification and development of a network of animal model testing facilities. The WHO is also developing a much-needed specimen repository and infrastructure for standardized testing of
Efforts are underway in several biotechnology companies to develop TB vaccine candidates, and some of the large vaccine manufacturers have maintained relatively low level but steady endeavors to explore potential candidates. Members of both large and small pharma are working on adjuvant development. It is expected that industry could contribute an enormous amount to TB vaccine development efforts, particularly although not exclusively, in production, efficacy trials, and ultimately vaccine manufacture and delivery. Because of the long-term commitment necessary, the fundamental knowledge that must still be acquired and the urgent need, industry cannot be expected to develop TB vaccines alone. Among other joint efforts, an international, public-private collaborative network of clinical efficacy trial sites will be needed. Such a network might be developed in whole or part through collaborations with international trial site infrastructures being established for AIDS and malaria vaccine testing.

As stated in the Blueprint for TB Vaccine Development: "Most importantly, successful development of effective tuberculosis vaccines will require a sustained commitment and extensive collaboration among participating governments, scientists, vaccine manufacturers and health care personnel both within the US and internationally, including developing and industrialized nations as full partners".

Progress towards these goals is being made. Recent scientific advances, combined with increasing political will by the US government (e.g., the President's Millennium Vaccine Initiative and March 2, 2000 White House meeting), have combined with new sources of support from private foundations and public funding agencies in the US and abroad. A consensus in the TB control and research communities has identified the crucial need for improved vaccines. There is widespread agreement that effective chemotherapy and effective immunization would have synergistic effects on ultimate control of the TB epidemic. Today is the most fortuitous time ever realized to develop effective TB vaccines. A joint public-private effort must be created to increase public awareness of the need for more effective TB vaccines and to impart a sense of urgency to the task of developing them.
References

15. WHO/TB/98.248
36. NIAID, National Vaccine Program Office and CDC’s Advisory Council on the Elimination of TB

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