Ann M. Ginsberg, MD PhD

# The TUBERCULOSIS

## Scientific Challenges and Opportunities

SYNOPSIS

ONE IN EVERY THREE people on Earth is believed to be infected with *Mycobacterium tuberculosis*, leading to seven to eight million cases of active tuberculosis (TB) per year and approximately three million deaths annually. This epidemic, like those of most infectious diseases, creates scientific challenges and opportunities as it raises the demand for public health solutions. The currently available weapons for fighting TB are inadequate. The ultimate goal of biomedical TB research is to lessen the public health burden of this disease by developing improved diagnostic, therapeutic, and intervention strategies. Achieving this goal requires a base of knowledge about the biology of *M. tuberculosis* and related mycobacteria, their interactions with human and animal hosts, and the nature of an effective host-protective immune response. TB researchers are applying this accumulating base of knowledge to developing rapid, easy-to-use diagnostic assays appropriate for low- as well as high-income countries, improving the current complicated therapeutic regimen, identifying potential new drugs to combat multidrug-resistant TB, and creating more effective vaccines.

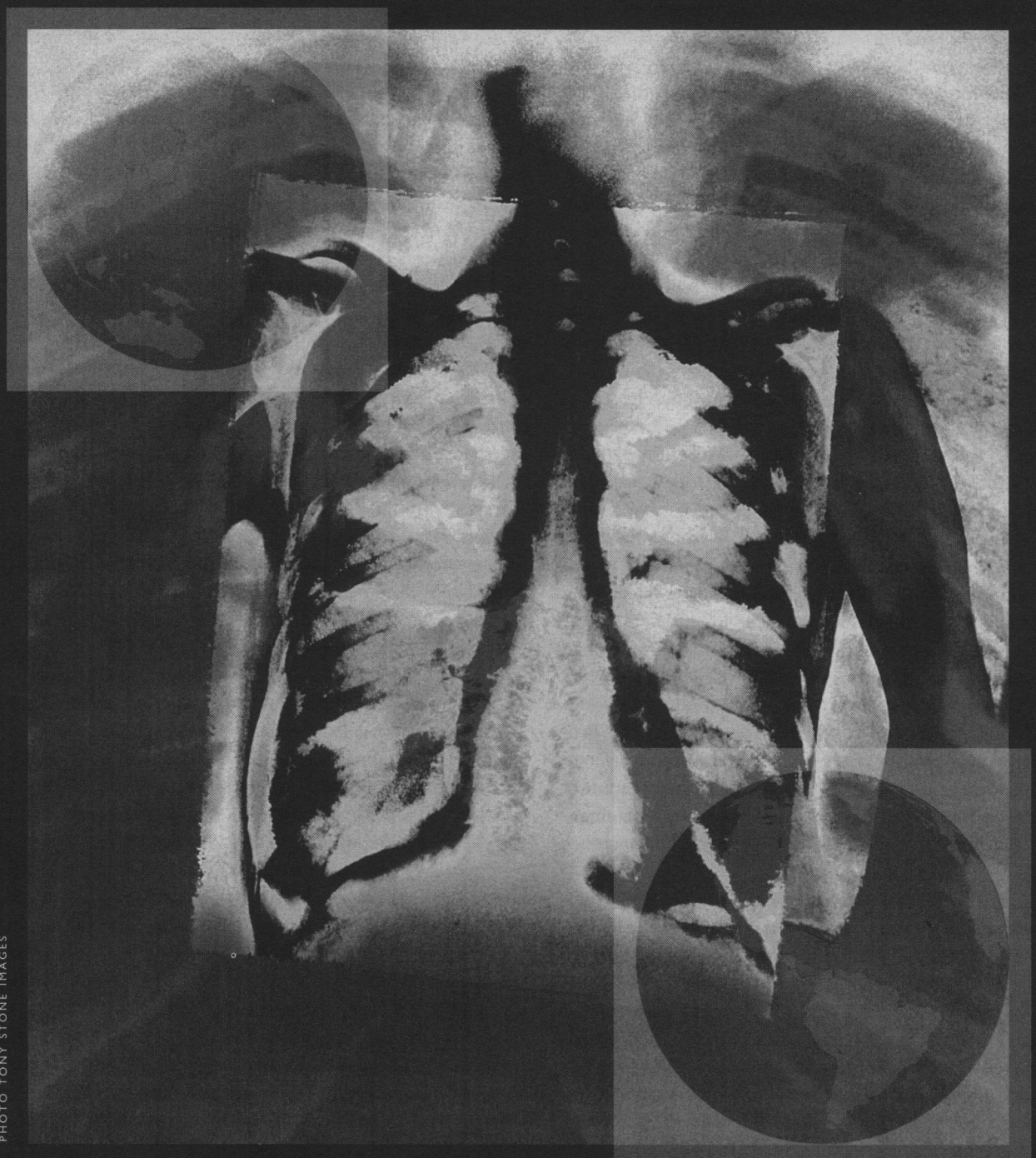
**IN 1991, a New York prison guard contracted a strain of tuberculosis (TB) that was resistant to all of the usual drug therapies, igniting a wave of media, public, and Congressional alarm over a growing TB epidemic in the United States.[1,2] The problem of TB had been considered solved after the introduction of effective antibiotics in the 1940s to 1960s. By the late 1980s, however, the number of cases was on the increase and new antibiotic-resistant strains were emerging, raising the prospect that TB might once again become incurable.**

Since it is spread through the air, TB threatens everyone, and pressure on Congress and the public health and biomedical research infrastructure to "solve the problem" became intense. How did Congress respond? In addition to dramatically increasing funds for control programs, Congress mandated that the National Institutes of Health (NIH) increase its budget for TB research approximately tenfold. The TB research budget of the National Institute of Allergy and Infectious Diseases (NIAID), the lead TB research institute at NIH, increased from approximately $3.5 million in 1991 to approximately $35 million in 1996. This article will highlight some of the research challenges and opportunities addressed with the help of this increased funding.

In 1996, the United States enjoyed its fourth consecutive year of declining TB case rates, recording 21,337 cases, the lowest number since 1985.[3] Yet, by 1996 the TB case rate had either stayed the same or increased in 23 states and the District of Columbia relative to 1995.[3] Between 1993 and 1996, multidrug-resistant TB was detected in a record high of 42 states and the District of Columbia, compared to only 13 states in 1991.[4]

The global incidence of TB was recently estimated to have been approximately 7.3 million cases in 1995,[5] causing approximately three million deaths

# EPIDEMIC



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per year, more than from any other single infectious disease. One-third of the world's population is believed infected with the causative bacterium, *Mycobacterium tuberculosis* (*M. tuberculosis*).[6] TB has become the leading cause of death in people infected with HIV.[6]

*M. tuberculosis* is spread from person to person by the respiratory route—in airborne droplets expelled from the lungs of a person with active TB disease who coughs, sneezes, or speaks. The bacteria typically take root in the pulmonary alveoli (air sacs of the lungs) of a newly infected person. In a healthy person, immune defenses normally barricade the infection behind a wall of cells, limiting spread of the bacteria. This "enclosed" infection is referred to as "latent TB" and can persist throughout a person's life, never causing symptoms or being transmissible to others. However, if a latently infected person's immune system becomes weakened—by HIV infection, malnutrition, aging, or other factors—the bacteria can become "reactivated" and begin to spread within the lungs (active pulmonary TB) or even to other tissues (miliary or extrapulmonary TB). When this happens, the person develops active TB disease and becomes infectious, perpetuating the transmission cycle.

In modern, sophisticated diagnostic laboratories, diagnosis of active pulmonary TB disease, the majority of adult TB, is at least a two-step process. First, *M. tuberculosis* infection of the lungs is identified, typically through a combination of sputum smear microscopy, nucleic acid amplification-based assays, and a sensitive culture-based method. In the second step, the laboratory determines the susceptibility of the patient's particular *M. tuberculosis* isolate to the standard TB antimicrobial agents so that appropriate therapy can be prescribed. All of these methods except sputum smear microscopy are unavailable to physicians in most parts of the world. As a result, in developing countries, drug susceptibilities typically cannot be determined, and a patient with TB that is resistant even to two or more of the standard drugs is still treated with the standard regimen. This practice allows potentially even more complicated patterns of drug resistance to develop. Providing coverage with what may be in essence only one effective drug at a time allows naturally occurring mutant bacteria that are resistant to that drug to predominate within the patient, and hence the patient's TB becomes even more drug-resistant.

The most effective weapon currently available to fight TB is directly observed treatment, short-course (DOTS). DOTS is a method of ensuring compliance with the complex but standard six-month, four-drug course of TB therapy; the method includes the requirement that trained personnel observe patients taking their medication during at least the first two months of treatment. Where it has been thoroughly instituted in local control programs, it has been remarkably effective—achieving over an 85% cure rate in a demonstration project in China, for example.[7] To date, however, only 10% of the world's TB patients, are estimated to have access to DOTS.[5] Because drug resistance develops due to partial and inadequate treatment, widespread use of DOTS would reduce the emergence of new multidrug-resistant strains of TB, but even widespread use of DOTS would not address the many cases of multidrug-resistant TB that already exist and the significant reservoir of future cases represented by people with latent multi-drug resistant infections. Approximately 40% of people with multidrug-resistant TB ultimately die of incurable TB.[4]

What can biomedical research contribute to solving these grave problems? The toolbox presently available to health care providers is woefully inadequate. In most parts of the world, it contains sputum smear microscopy for *diagnosis*, a six-month or longer course of *therapy* with first-line drugs (isoniazid, rifampin, pyrazinamide, streptomycin, ethambutol, and thiacetazone), and Bacille Calmette-Guerin (BCG), a *vaccine* of at best variable efficacy against pulmonary TB, the most common form of the disease. Even in industrialized nations, little more is available: diagnosis can be accomplished more rapidly through relatively expensive and technically sophisticated assays, and costly drugs are available for the treatment of multidrug-resistant TB.

Better tools for fighting TB are vital to the long-term control and elimination of TB as a major public health burden in the United States and globally. Their development depends on a greater foundation of knowledge about *M. tuberculosis* and how it interacts with the human host. Advances in biotechnology, including automated DNA sequencing, are contributing to breakthroughs in our understanding of TB and to the development of improved diagnostics, therapies, and vaccines.

## Diagnostic Assay Development

The traditional tuberculin skin test to detect *M. tuberculosis*-infected individuals (with latent or active infections) is useful for general surveillance only in countries such as the United States and the Netherlands, where TB is not endemic and BCG vaccines are not widely administered (people vaccinated with BCG often have positive skin tests).

Sputum-smear microscopy of Ziehl-Neelsen stained specimens, the method used for diagnosing TB in the developing world, is relatively low-tech, labor-intensive, and insensitive (at least 10,000 tubercle bacilli per ml of sputum are needed for a positive reading). Furthermore, the Global TB Programme of the World Health Organization estimates that only 35% of all people infected with *M. tuberculosis* are sputum-smear positive.[5] A fast, inexpensive, easy-to-perform, sensitive, and specific diagnostic test for TB would be of immense value worldwide. The

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knowledge necessary to design such an assay is being developed by researchers studying the human immune response to *M. tuberculosis* infection. They are identifying, for example, antibodies produced in response to infection and during active disease in children and adults including people who are HIV-negative and HIV-positive.

Identifying the key antibodies produced in infected individuals could lead to the development of a blood test that might employ a dipstick or another similarly easy-to-use method (Personal communication, Maria L. Gennaro, MD, Public Health Research Institute).[8,9] In another approach, researchers have begun using the ability to manipulate mycobacteria-specific bacteriophages (bacteriophages are viruses that infect bacteria) to create diagnostic tests in which growing *M. tuberculosis* bacteria literally light up. This technology is being applied to creating a Polaroid film-based, simple and inexpensive rapid TB diagnostic and an inexpensive, low tech, rapid method for determining drug susceptibilities.[10] If successful, this diagnostic would for the first time enable health care practitioners in low-income countries to appropriately tailor TB therapy for drug-resistant strains.

TB epidemiology and surveillance are also becoming more sophisticated, as researchers develop increasingly precise tools for identifying and tracking specific strains based on knowledge of the molecular genetic structure and DNA sequence of *M. tuberculosis*. In the last few years, for example, restriction fragment length polymorphism (RFLP) analyses based on use of the *M. tuberculosis* insertion element IS6110 sequences as a probe have facilitated epidemiologic research and investigations of outbreaks.[11] Epidemiologists have used this approach, for example, to track the spread of a single strain of multidrug-resistant TB across the United States[12] and to identify groups of people in San Francisco at high risk of contracting multidrug-resistant TB.[13] The results of such studies can lead to new, improved TB control strategies by helping efforts to be targeted more precisely.

## DRUG DEVELOPMENT

The need for new TB drugs is evident. Ideally, one would like a single-dose antibiotic that cures active disease and prevents reactivation of latent infection. Short of this dream, a safe drug that would shorten the necessary course of therapy or simplify the current complicated regimen would make a major contribution to TB control. Similarly, new classes of drugs that are inexpensive and easy to administer that could be used in treating multidrug-resistant strains would add important weapons to the anti-TB arsenal.

**Global mortality from TB is now estimated at almost three million people per year, higher than from any other single infectious disease, and one-third of the world's population is believed infected with...*M. tuberculosis*.**

Investigators are approaching these challenges in several ways. Some are identifying potential new drug classes by elucidating the mechanisms of action of key mycobacterial-specific enzymes and designing or selecting inhibitors;[14–17] others are screening large numbers of available compounds in *in vitro* and *in vivo* (animal) models set up to mimic human TB as much as possible,[18,19] while still others, especially in industry, are using combinatorial chemistry and high throughput assays to identify possible novel leads.[20] Simultaneously, human trials are underway to evaluate new rifamycin derivatives that have a longer

half-life than rifampin, which would allow them to be given less frequently than the drugs used in current standard regimens. Based on growing knowledge of the human immune response to *M. tuberculosis* infection, immunomodulators such as the cytokine interleakin-2 (a cytokine is a hormone secreted by cells of the immune system that helps regulate the immune response) are also being evaluated for their ability to improve the efficacy or shorten the duration of current therapy (Personal communication, Gilla Kaplan, PhD, Rockefeller University).

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## VACCINE DEVELOPMENT

Development of improved vaccines is critical to the ultimate control and elimination of TB in the United States and worldwide. The practical barriers to controlling TB on a global scale through DOTS, the spread of multidrug-resistant TB, the enormous global burden of the TB epidemic, the growing TB-HIV co-epidemic in many regions of the world, and the relative inefficacy of current BCG vaccines underlie the need for a new, better vaccine. Vaccine development, while of the highest priority in TB research, represents one of its greatest challenges.

In the last few years, numerous investigators have developed literally dozens of potential vaccine candidates, representing a variety of approaches. They include: modified BCGs; live, attenuated strains of *M. tuberculosis*; non-pathogenic, environmental mycobacteria; subunit vaccines; and naked DNA vaccines.

**BCG vaccines.** *M. bovis* is a mycobacterium closely related to *M. tuberculosis* that causes tuberculosis in cows and in humans ingesting unpasteurized milk from infected cattle. The first BCG vaccine was developed at the Institut Pasteur between 1906 and 1919 by deriving a non-disease-causing (or "attenuated") strain of *M. bovis* and was first used in humans in 1921. Since 1921, over three billion doses of BCG have been administered worldwide; in 1996, 100 million infants were immunized with BCG.[21]

BCG is one of the six basic antigens authorized for use under the World Health Organization's Expanded Programme for Immunization (EPI). The Netherlands and the United States are the only two countries that currently do not recommend universal BCG vaccination of children.

Several recent meta-analyses of BCG vaccination studies show wide variability in BCG's ability to provide protection against TB. Against miliary TB and TB meningitis in children, BCG's reported efficacy ranges from 46% to 100%.[22–24] However, against pulmonary TB, which represents the majority of the burden of this disease, BCG's reported efficacy ranges from 0 to 80%.[22–24] Numerous factors have been cited as possible explanations for this variability, including: methodological differences among the studies; variations among BCG strains cultured in laboratories around the world; differences in the strains of *M. tuberculosis* found in different parts of the world; nutritional factors; environmental factors; varying exposure to non-pathogenic mycobacteria found in varying extents in the soil and other environmental niches around the world; and genetic differences among host populations. Most likely, a combination of factors is responsible for the variable efficacies observed, with differences in BCG strains and varying exposure to environmental mycobacteria perhaps playing the strongest roles.

**The past few years have seen remarkable productivity in the development of potential vaccines in the laboratory and in testing these candidates in animal models.**

Historically, manufacturers of BCG vaccines have selected for strong delayed type hypersensitivity (DTH) response on the tuberculin skin test while trying to decrease any potential for adverse reactions such as skin ulceration, believing that DTH response correlated with protective immune response. More recent evidence suggests that a strong DTH response does not necessarily reflect a strong protective immune response. A recent report presents evidence that the efficacy of any given strain of BCG decreased as its passage number (the number of times a sample is serially cultured) increased, although the DTH response remained high.[25] While raising an important point about current BCG vaccine strains, this report does not address why a single BCG preparation

was shown to be highly efficacious in protecting against TB in one part of the world while providing little or no protection in another.[26] Therefore, while probably a contributing factor, serial passage of BCG strains with selection for decreased adverse reactions but retention of skin test conversion cannot represent the entire explanation for the variable protection against pulmonary TB observed in different studies. The efficacy of BCG vaccination appears to vary with geographic latitude—the farther from the equator, the more efficacious the vaccine. Non-pathogenic environmental mycobacteria are more prevalent closer to the equator. Presumably, exposure to these mycobacteria induces a degree of cross-protective immunity in the exposed human populations so that any potential protection from BCG is masked.

On the positive side, BCGs provide relatively good protection against extrapulmonary TB in children and have demonstrated efficacy against leprosy, another mycobacterial disease.[27] As a result, it is difficult to imagine simply replacing BCG with a potentially better TB vaccine. Rather, in most parts of the world, new, hopefully more efficacious, TB vaccines will have to be tested and administered in addition, or subsequent, to BCG vaccination.

**Modified BCGs.** Recombinant DNA methodologies have been used to add one or more protective antigens or cytokines to BCG to boost its protective effect.[28,29] This approach takes advantage of BCG's ability to persist intracellularly in the host, mimicking *M. tuberculosis* infection, and its endogenous ability to stimulate a human generalized immune response. To date, several modified, recombinant BCGs have been developed that show protective efficacy in either mice or guinea pigs or both similar to, but not significantly better than, a currently used strain of BCG.[30]

BCG has also been modified through the creation of auxotrophic mutants (auxotrophic mutants are mutants that require the addition of a specific amino acid to their growth media in order to be viable; in the absence of this amino acid the mutant dies, thereby potentially making it safe for human administration).[31,32] Currently, in some countries where BCG is otherwise administered universally, health authorities recommend that HIV-positive people, including children, not be BCG vaccinated, due to the possibility of causing disseminated BCG infection in these immunocompromised hosts. Researchers have reported that some auxotrophic BCG strains induced an appropriate host immune response in mice and were ultimately cleared from the body. Even immunocompromised (severe combined immunodeficiency disease) mice survived long-term, suggesting that these mutants were safe for immunocompromised mouse hosts.[33] Auxotrophic strains of *M. tuberculosis*, in contrast, killed immunocompromised mice, indicating that further attenuation (lessening of pathogenicity or virulence) of *M. tuberculosis* would be necessary before such vaccine candidates could be tested in immunocompromised humans.

**Attenuated strains of *M. tuberculosis*.** Sequencing of the entire *M. tuberculosis* genome—both a laboratory strain (H37Rv) and a recent, highly transmissible, clinical isolate[34] is virtually complete. H37Rv is being sequenced at the Sanger Centre in England (Personal communication, Bart Barrell, PhD, Sanger Centre) and the clinical isolate by the Institute for Genomic Research in Gaithersburg, Maryland (Personal communication, Robert Fleischmann, PhD, Institute for Genomic Research). It is hoped that this information, combined with recently developed techniques for mutating the *M. tuberculosis* genome[35,36] and analyzing these mutants in animal models of TB will enable relatively rapid advances in identifying which *M. tuberculosis* genes contribute to this pathogen's virulence and in understanding how they do so. Such knowledge would make it possible to rationally create strains of avirulent (non-disease-causing) *M. tuberculosis* as the basis for vaccine candidates (see also "Genomics," below).

Another approach, already underway, to identifying genes that contribute to the virulence (disease-causing capacity) of *M. tuberculosis* is the determination of genetic differences between BCG (which is non-pathogenic) and a virulent (or pathogenic) strain of *M. bovis*. These experiments have identified three regions of difference between BCG DNA and virulent *M. bovis* DNA. One of these regions, termed RD1, was absent from all BCGs tested but present in all clinical isolates of *M. bovis* and *M. tuberculosis* examined.[37] More detailed examination of RD1 may reveal a gene or genes whose functional deletion would attenuate *M. tuberculosis*. Alternatively, one can imagine creating and testing a recombinant BCG that expresses part of the RD1 sequence, with the hope that such a recombinant BCG might induce stronger protection than BCG itself while retaining the avirulent nature of BCG. Work in this area should progress rapidly over the next couple of years.

**Subunit vaccines.** Subunit vaccines—comprised of a subset of *M. tuberculosis* antigens instead of the whole bacterium—that could induce a strong protective immune response would be attractive because of their likely relative safety as compared to a whole bacterium and potential ease of manufacture. Several investigators are trying to develop TB vaccine candidates of this type, based on a variety of "culture filtrate proteins" (CFPs)—that is, complex, relatively poorly defined mixtures of mycobacterial proteins that are thought by investigators to stimulate the host-protective immune machinery during infection.[38] While some investigators are testing the ability of CFP preparations to induce a protective immune response in mice and guinea pigs, others are testing individual proteins

or well-defined mixtures of a small number of purified proteins. Encouraging results have been seen in mouse and guinea pig experiments in which animals vaccinated with a CFPpreparation of *M. tuberculosis*, combined with adjuvant and appropriate cytokines, were protected from a challenge with *M. tuberculosis* at least as well as animals vaccinated with BCG.[38]

Instead of basing vaccine candidates on preparations of whole proteins, some investigators are trying to identify peptide antigens (specific pieces of protein made up of a few amino acids) that could evoke a protective immune response. Recent studies have shown that combinations of several such peptides are likely to be more effective than any individual peptide in stimulating a protective host immune response. Prediction of some types of immune-stimulating peptides is now possible through computer algorithms,[39] and efforts are underway to annotate the completed *M. tuberculosis* genomic sequence using such computer-based predictions (Personal communication, Anne De Groot, MD, Brown University, and Robert Fleischmann, PhD, Center for Genomic Research). These studies provide an obvious example of the potential for rapid advances made possible by identifying the entire DNA sequence of a microbial pathogen.

A few nonprotein antigens are currently under investigation as a potential basis for TB vaccines. Mycobacterial cell wall mycolic acids[40] and carbohydrates[41] are both being investigated for their ability to induce a protective immune response. More extensive work in this area is warranted.

**DNA vaccines.** A recent major advance in vaccine development has been the recognition that immunization with naked DNA that codes for appropriate antigens can confer protection against a variety of bacterial, viral, and parasitic pathogens.[42] (Naked DNA is DNA itself without associated proteins or any other components of the microbial pathogen.) The potential efficacy of DNA vaccination against TB has been demonstrated by a number of research groups.[43–45] DNA vaccination offers several advantages over complex bacterial protein preparations and live, attenuated strains of mycobacteria, including enhanced safety and relative ease and low cost of manufacture. As a result, various approaches are currently being explored for increasing the amount of antigen produced from the DNA vaccine once it is inside the host cells and thereby potentially icreasing the ability of DNA vaccines to stimulate a protective host response. Attempts are also being made to enhance immunogenicity by co-expressing cytokines or altering the adjuvant(s) (compounds that enhance the immune response) used. Some candidate DNA vaccines are now being tested for their ability to confirm long-term protection against *M. tuberculosis* in both the guinea pig and mouse.

Some investigators are taking advantage of knowing the entire DNA sequence (genome) of *M. tuberculosis* to try to identify all of the protein antigens of this bacterium that can stimulate a protective immune response. One technique, pioneered by Stephen Johnston, PhD PhD, University of Texas, Southwestern, is known as "Expression Library Immunization."[46] It attempts to screen the whole genome, using an animal challenge model to find those "pieces" that encode protective antigens. Such antigens, once identified, could form the basis of either a protein subunit vaccine or a DNA-based vaccine.

**Nonpathogenic mycobacteria.** Several mycobacteria that do not typically cause disease in humans have been considered as the potential basis for TB vaccines, including *M. habana* (which has been isolated from monkeys),[47] *M. microti* (which causes TB in voles and other small mammals),[48] and *M. vaccae* (which has been isolated from Ugandan soil).[49] Most recently, *M. vaccae* has been evaluated as an immunotherapeutic agent—rather than as a vaccine candidate—in several trials, including a recently completed efficacy (Phase III) trial in Durban, South Africa (Personal communication, John Standford, MD, and Graham Rook, MD, Stanford and Rook Ltd.), and an NIAID-supported safety/immunogenicity (Phase I/II) trial in Kampala, Uganda (Personal communication, Jerrold Ellner, MD, Case Western Reserve University). To date, the preponderance of evidence suggests that *M. vaccae* is not an effective immunotherapeutic for TB, at least when administered in a single dose in combination with standard anti-TB drug therapy.

*M. vaccae*, a nonpathogenic, soil mycobacterium, and *M. smegmatis*, a relatively rapidly growing, nonpathogenic mycobacterium, are also being used in candidate vaccines, as tiny "factories" that make large amounts of one or a few protective mycobacterial antigens.[38] In addition, researchers are exploring the potential of using non-mycobacterial, non-disease-causing microbes, such as strains of the *Salmonella* bacterium and *Vaccinia* virus, to produce large quantities of protective mycobacterial antigens within host cells.

**Challenges for vaccine developers.** A number of significant challenges face those interested in developing improved TB vaccines. Many of these are issues related to the design of clinical trials—such as defining what we would hope a vaccine candidate will accomplish: inhibit initial infection, block transmission, prevent primary disease, or block reactivation of latent infection. For example, a vaccine designed for delivery to teenagers or young adults who have already had BCG vaccinations or have been exposed to *M. tuberculosis* or both could be tested in areas where the disease is endemic in a much shorter time frame than a vaccine meant to replace BCG for use in newborns (which would potentially require decades of fol-

low-up to assess its efficacy). The available animal models must be modified to address these different goals. Models of "latent" infection and disease reactivation are under active development in several laboratories.

Another critical need is to identify and validate correlates, or easily measured markers, of the human protective immune response. Without such markers, vaccine trials will require decades of study subject follow-up given that many people first develop active disease years after infection with *M. tuberculosis*. This is an area of active investigation. A related issue is that none of the available animal models necessarily replicates the human immune response. Animal models can therefore be used to provide some indication of how safe a new vaccine is and how effective it is relative to BCG (for what this comparison is worth, given the limitations of BCG vaccination in humans), but any true indication of efficacy will be dependent on the results of human trials.

The past few years have seen remarkable productivity in the development of potential vaccines in the laboratory and in testing these candidates in animal models. Active discussions are underway within the research and TB control communities as to how best to design clinical trials of the most promising potential vaccines. The challenge for the next few years includes furthering our basic understanding of the human host response to *M. tuberculosis* while applying this knowledge to rationally prioritizing candidates and designing both the trials and the infrastructure for human clinical testing.

## GENOMICS

Most auspicious of all among research advances is that the DNA sequence of the entire *M. tuberculosis* genome has been determined and is publicly available. Although microbiologists and infectious disease specialists won't fully understand what the sequence means for many years, it immediately gives them the complete blueprint of *M. tuberculosis* from which to work. For TB researchers, having this cache of information would be equivalent to the first Explorer mission having available upon landing a complete geological survey map of Mars. Now, instead of painstakingly examining one gene or one protein at a time, investigators will be able to look at once at all of the genes being "turned on" or expressed under any given set of experimental conditions. They can thereby take "whole genome" approaches to exploring gene expression, identifying novel targets of drug action, developing diagnostic tools, and revealing disease-causing mycobacterial genes or proteins and protective antigens as potential foundations for new vaccine candidates.

Innovative technologies are already being developed to allow scientists to think and work on this much grander, more efficient scale.[50–52] For example, micro-arrays (microscope slide-sized platforms containing thousands of segments of DNA representing either the whole expressed DNA sequence or the entire genome) allow researchers to ask two new kinds of questions, essentially performing the equivalent of hundreds or thousands of experiments at once: What genes in *M. tuberculosis* are "turned on" or expressed under a given set of experimental conditions, for example, at a particular temperature or oxygen concentration or pH? What genes in a particular clinical isolate of *M. tuberculosis* are mutated (have differences in their DNA sequence) relative to a reference strain?

The availability of the entire DNA sequence of *M. tuberculosis*, the development of techniques for using these data, and the resulting answers to both fundamental and applied questions are already beginning to change the landscape of TB diagnosis, treatment, and vaccine development. The full impact will probably not be felt for several decades, but the hope is that, in the not too distant future, we will look back on today's management of TB as truly being in the "Dark Ages."

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**Dr. Ginsberg is Program Officer for Tuberculosis, Leprosy, and Other Mycobacterial Diseases with the Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health.**

Address correspondence to Dr. Ginsberg, Solar Building, Rm. 3B06, 6003 Executive Blvd., MSC 7630, Bethesda MD 20892-7630; tel. 301-496-5305; fax 301-496-8030; e-mail <ag73i@nih.gov>.

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