Tuberculosis and Leprosy Panels

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Guidelines

Tuberculosis and Leprosy Panels

Research areas of special relevance to tuberculosis and leprosy are the molecular genetics of virulence and pathogenicity; drug targets and mechanisms of resistance; improved animal and human models of protective immunity; mycobacterial constituents that induce pathogenetic and protective cytokines; and the molecular epidemiology of tuberculosis and leprosy. Development of a better vaccine is the highest priority.

The goal of the Panels is to foster research in the following areas:

1. Molecular genetics of *Mycobacterium tuberculosis* and *Mycobacterium leprae*

    - Molecular basis for virulence and pathogenicity
    - Mechanisms of drug action and drug resistance
    - Mapping and sequencing of the genomes

2. Immunobiology and pathogenesis of tuberculosis and leprosy

    - Cells and cytokines involved in pathogenesis and protective immunity
    - Antigen(s) in mycobacteria that evoke protection
    - Mycobacterial products that induce cytokine production and possess adjuvant properties
    - Tuberculosis and leprosy in persons co-infected with HIV
    - Immunologic mechanisms of the spectrum of leprosy and reactions
    - Molecular basis of neurotropism of *M. leprae* and immunologic mechanisms in leprous neuropathy
    - Genetically determined human resistance and susceptibility to tuberculosis and leprosy

3. Development of improved animal and cell-culture models

    - Models resembling human infection and natural history of disease
    - Genetic manipulation of experimental animal models for the study of immunity
    - Improved in vitro models for studying interactions of host lymphocytes and macrophages with mycobacteria

4. Clinical and epidemiologic application of basic technology

    - Early diagnosis of tuberculosis and leprosy and identification of drug-resistant disease
    - Immunologic intervention for intractable tuberculosis and combined infection with *M. tuberculosis* and HIV
    - Molecular epidemiology of tuberculosis and leprosy
    - Potential targets for drug development
    - Development of a more specific and more sensitive skin test and serodiagnostic reagents for tuberculosis and leprosy
    - Application of progress in genetic and immunobiological studies for development of a potent vaccine for tuberculosis and leprosy

Five-Year Summary

Panel Consolidation

In 1996, after considerable discussion, the previously separate Panels for tuberculosis and leprosy were combined to form a joint Tuberculosis and Leprosy Panel. The operating principles and scientific goals were reviewed at the Panel meetings and via mail, with full input and agreement from the members of both Panels. The number of Panel members was decreased from nine each to six each for the U.S. and Japanese Panels, and there was one chair for each Panel, in 1996-1999. Beginning in 2000, the U.S. Panel and the Japanese Panel each consists of a single chair and five members.

Priority Scientific Areas

The categories of research identified by the Panels as priority areas for the 5-year period 1996-2000 are as follows:

1. Molecular genetics

- Virulence
- Pathogenicity
- Mechanisms of drug action and development of resistance
- Mapping and sequencing the genomes of *M. tuberculosis* and *M. leprae*

2. Immunobiology and pathogenesis

- Cells and cytokines involved in protective immunity
- HIV infection and tuberculosis
- Identification of antigens in mycobacteria that evoke protection
- Mycobacterial products that induce cytokine production
- Adjuvant properties of natural and synthesized mycobacterial products

3. Development of improved animal and cell-culture models

- Development of animal models resembling human infection
- Genetic manipulation of experimental animal models for the study of immunity
- Improved in vitro models for studying T cells and macrophages and their interactions with mycobacteria

4. Clinical applications of modern technology

- Early diagnosis
- Immunologic interventions for tuberculosis in HIV-infected persons
- Molecular epidemiology
- Identification of potential new drug targets

Overview of Research

The relative scientific interest in tuberculosis and leprosy continues to reflect the burden presented globally by these two diseases. Leprosy research is in many ways a victim of its own success. Effective drug regimens have brought about a striking decrease in the prevalence of leprosy, although it is thought that the incidence has not changed. As a consequence of the reduction in the global burden of leprosy, there has been a similar reduction in scientific research focusing on this disease. Nevertheless, the genome of *M. leprae* has been sequenced, setting the stage for an accelerated investigative effort. The challenge confronting those who make decisions about leprosy research is to heighten interest and funding in the face of the presumption that the information required for control and perhaps elimination of the disease is already known.

The opposite situation is true for tuberculosis. The resurgence of tuberculosis in the late 1980s and early1990s led to recognition that new tools were needed to combat the disease. Funding for tuberculosis research increased dramatically in the first half of the 1990s, leading to a number of fundamental scientific advances, including sequencing of the genome of a laboratory strain of *M. tuberculosis* and of a virulent clinical isolate of this organism. Even in the face of decreasing rates of tuberculosis in the United States and consistent funding for tuberculosis research at the National Institutes of Health, globally there is a vastly increased recognition of the need for new tools to apply in controlling the disease. This recognition has led to accelerated interest in tuberculosis research which, in turn, has been fueled partly by several initiatives in both the public and private sectors. These initiatives relate to development of vaccines, diagnostic tests, and drugs for treatment of tuberculosis. In highlighting the need for new tools to eradicate tuberculosis in the United States and to control the disease globally, the Institute of Medicine's recent report on elimination of tuberculosis in the United States focuses attention on the importance of tuberculosis research.

In view of the recent scientific advances and the increased interest in and enthusiasm for tuberculosis research, the next 5 years (2001-2005) should see major new developments that could result in better interventions for control of the disease.

Progress and Accomplishments

Major scientific progress in mycobacterial sciences has been made during the past 5 years. The door to rapid scientific advances has been opened by the sequencing of the genomes of one strain of *M. leprae* and two strains of *M. tuberculosis* (the avirulent H37Ra strain and the virulent H37Rv strain). Additionally, the availability and applicability of DNA microarrays is enabling rapid exploitation of genomic information and generation of detailed information about the dynamics of gene activation and expression, as well as comparisons of the genomes of various mycobacteria. For example, comparison of the genomes of the two strains of *M. tuberculosis* detected at least three distinct regions of variation. However, complementation of the avirulent strain with a fragment present in the virulent strain did not increase the virulence of the avirulent strain. Although this study did not identify genes responsible for the phenotypic differences in the strains, the approach is expected to enable identification of factors that are at least associated with virulence and that suggest new vaccine targets.

DNA microarrays have also been used to identify genetic differences among *Mycobacterium bovis* and strains of *M. bovis*-bacille Calmette-Guérin (BCG) that are used as vaccines. Distinct differences were shown, indicating that BCG has undergone genetic evolution that may account for the differences in protective efficacy in clinical trials. These findings have the potential to yield the ideal genomic structure for an improved BCG vaccine for tuberculosis.

There have been other advances in vaccine development. Both single proteins and mixtures of proteins from culture filtrate have been shown to have immunizing potential. Similarly, DNA vaccines encoding several immunogenic proteins have been identified. Recombinant BCG strains and auxotrophic strains of *M. tuberculosis* have also been developed and examined in animal models. The development of animal models (mouse, guinea pig, and rabbit) that allow evaluation of greater numbers of compounds has greatly facilitatesd the testing of candidate vaccines and has served to elucidate aspects of the host immune response. Dozens of candidate vaccines have been screened for toxicity and protective efficacy.

Surprisingly, evaluations of the effects of BCG vaccine in Malawi have shown that, although the vaccine provided no protection against tuberculosis, the incidence of leprosy was reduced.This effect may account for the previously noted global decrease in leprosy.

Underlying much of the progress in vaccine research has been the development of a more detailed understanding of the immune response to *M. tuberculosis* and the immunopathogenesis of the disease. Study findings have substantiated that interferon α and interleukin 2 (IL-2) play key protective roles, and scientists have carefully examined the effects of tumor necrosis factor-(TNFα), both in promoting formation of granulomas and in mediating the local and systemic effects of tuberculosis. Studies have also demonstrated the role of transforming growth factor β (TGF-β) in suppressing the response to *M. tuberculosis.*

With regard to the pathogenesis of leprosy, it has been shown that invasion of Schwann's cells by *M. leprae* is mediated by a specific interaction between a surface protein of the organism, ML-LBP21, and native laminain of the Schwann's cell. This observation provides important insights into the basis of the neurotropism of *M. leprae*.

Substantial progress has also been made in elucidating the genetic basis of drug resistance and the mechanisms of drug action. The mutations that account for nearly all instances of resistance to rifampin have been identified, as well as mechanisms for most instances of resistance to isoniazid, ethambutol, streptomycin, pyrazinamide, and the fluoroquinolones. Similar if not identical mutations have been associated with resistance to rifampin and fluoroquinolones in *M. leprae*. In addition, the mechanism of resistance to dapsone in *M. leprae* has largely been determined. Understanding the genetics and mechanisms of drug action has provided the foundation for approaches to rational drug design that are being implemented.

Several new categories of drugs with antituberculosis effects have been identified and are in various stages of testing. These include the oxazolidinones and the nitroaimidazoppyrans. New compounds from older categories of antimycobacterial agents, especially the rifamycins and the fluoroquinolones, are being studied, and many analogues of ethambutol are being evaluated. The first new drug specifically for tuberculosis in nearly 30 years, rifapentine, was approved by the U.S. Food and Drug Administration in 1998.

All of these studies to develop drugs for treatment of tuberculosis are greatly facilitated by the recent availability of high-throughput assays that are based, for example, on incorporation of luciferase genes into the test strains of *M. tuberculosis* that render the bacteria bioluminescent and on the use of fluorescent molecular probes (molecular beacons). Both of these methods

provide rapid assessment of cell viability and, hence, of drug effect. The use of radiorespirometry has also enabled the screening of large numbers of compounds for antileprosy effects.

Advances in diagnostic technologies have related mainly to refinements in rapid amplification techniques to identify *M. tuberculosis* and to detect drug resistance by detection of mutations that are associated with a resistant phenotype. Before 1999, methods of nucleic acid amplification to detect *M. tuberculosis* had been approved only for sputum sediments that are "smear positive" for acid-fast bacilli. Recently, however, on the basis of data showing improved sensitivity, the MDT (*Mycobacterium tuberculosis* Direct test) was approved for use for "smear-negative" sputum sediments. Both luciferase expression and molecular beacons are being explored for use in the detection of drug resistance.

A commercial test for detection of interferon g in whole blood is in clinical trials. Preliminary data on the antigen currently under study are not promising, but the use of other antigens is being explored.

Epidemiologists continue to use the insertion sequence IS6110 in the molecular epidemiology of tuberculosis. Refinements are being examined with the intent of speeding the ability to genotype organisms. One such refinement is spacer oligonucleotide typing, which is based on polymorphism of the direct repeat locus in *M. tuberculosis*.

Future Goals

At their meeting in Yokohama, Japan, in July 2000, the U.S. and Japanese Panels will discuss the priorities for the next 5-year period. It is anticipated that the scientific target areas will remain much the same as those for the past 5 years. However, the availability of the genome sequences will provide a substantial impetus in both tuberculosis and leprosy research.

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