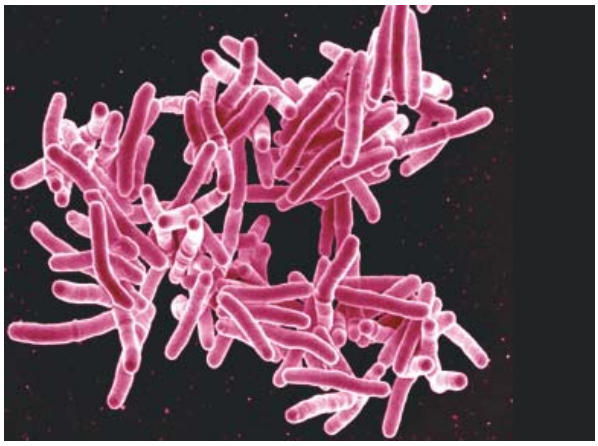
TUBERCULOSIS AND LEPROSY PANELS

[Established as separate Leprosy Panels and Tuberculosis Panels in 1965; amalgamated and renamed in 1996]

In 1965, separate Panels on Leprosy and Tuberculosis were established as two of the five original USJCMSP panels. By 1996, however, interest in leprosy was waning and, after much discussion and planning, the panels were amalgamated and renamed the Tuberculosis and Leprosy Panels. Both diseases are caused by mycobacteria, and it was thought that advances made in one field could help stimulate the other, a prediction that has since become true. Also, the amalgamation of the Leprosy and Tuberculosis Panels was part of a larger reorganization of the U.S.–Japan Program, and it removed financial constraints on the formation of the new Japanese and U.S. Acute Respiratory Infections Panels, which also occurred in 1996.

At their annual meeting in Kona, Hawaii, held January 19–20, 1996, members of the USJCMSP Joint Subcommittee on Program Review and Planning defined new operating principles and scientific goals for the merged Leprosy and Tuberculosis Panels. When the Panels merged, they entered a five-year transition period. During that time, the merged Japanese and U.S. Panels each had two co-chairmen and four panel members. However, prior to the amalgamation, the Tuberculosis and Leprosy Panels had organized their annual research conferences in the same venue on the same date, and had devoted one day to a joint meeting. This traditional way of organizing the research conferences of the Tuberculosis and Leprosy Panels contributed to their smooth amalgamation.

"When the Panels for TB and Leprosy merged, they initially had two chairmen," Dr. Tadao Shimao said in an August 2004 interview. Dr. Shimao, who helped guide the amalgamation, was Chair of the USJCMSP Japanese Delegation from 1993–2001, and a Delegation member from 1977–1993. Since the amalgamation of the Leprosy and Tuberculosis Panels, the USJCMSP remains the only high-visibility, international organization that continues to emphasize the study of leprosy.



Mycobacterium tuberculosis, the cause of tuberculosis

"When the U.S.–Japan Program began, we knew nothing about the molecular biology of tuberculosis," said Dr. Shimao. "Now we can study tuberculosis at the subcellular and molecular levels. In the forty years of the Program, much scientific progress has been made."

Today, the Tuberculosis and Leprosy Panels contribute in important ways to the goals of the USJCMSP. A major reason for their current success stems from productive collaborations between U.S. and Japanese investigators. The joint annual scientific meeting of the Tuberculosis and Leprosy Panels, which alternates between the two countries, helps facilitate the creation of joint funding opportunities, exchanges of personnel between laboratories, and literally scores of publications. The Panel's activities bring together groups of investigators with expertise in several areas, including: (a) the molecular epidemiology of leprosy and tuberculosis; (b) mechanisms of drug resistance in mycobacteria; (c) the immunology and cytokine biology of tuberculosis and leprosy in humans and animal models; and (d) the preclinical evaluation of new tuberculosis vaccines. Thus, the Tuberculosis and Leprosy Panels continue to fulfill the USJCMSP's mandate to foster bi-national research on the two diseases, to promote the training of a new cadre of scientists, and to generate

new knowledge with application to disease-control strategies.

Scientific progress, particularly in the study of tuberculosis, has been rapid since the 1980s, when more research funds became available and allowed the application of new technologies for sequencing the genomes of pathogens, and then analyzing and comparing their genomes. These efforts are beginning to yield critical information about the biology and pathogenesis of many organisms, including the mycobacteria that cause tuberculosis, leprosy, and other diseases. Meetings sponsored by the USJCMSP on interdisciplinary topics are further stimulating this research.

"Inter-panel meetings and conferences are now getting more attention, because it is clear that scientists in one area have to reach out to scientists in another," said Dr. Adel Mahmoud in a June 2004 interview. Dr. Mahmoud, who is president of Merck Vaccines at Merck & Co. in New Jersey, became a member of the U.S. Delegation in 1994 and has served as its Chair since 2001. "A good example is that the Tuberculosis and Leprosy Panels have met together with the Immunology Boards." Another example of interdisciplinary collaboration, Dr. Mahmoud said, is that USJCMSP-sponsored conferences on topics such as genomics or vaccine development pull together experts from multiple research fields and encourage the exchange of state-of-the-art information about leprosy, tuberculosis, and a host of other infectious diseases.

Leprosy. Forty years ago, when the original USJCMSP Leprosy Panels began their work, an estimated 15 million cases of leprosy occurred worldwide. An early focus of the USJCMSP was to develop ways to control the disease, which is caused by *Mycobacterium leprae*, and members of the Leprosy Panels contributed to the design of several drug regimens. In the early 1980s, multiple-drug therapy (MDT) for leprosy was introduced, which caused a dramatic decline in the prevalence of the disease.

Approximately 700,000 to 800,000 new cases of leprosy now occur globally each year.[1] The number of new cases has remained stubbornly constant for about 15 years, which underscores the need to understand more completely how the leprosy bacterium is incubated and transmitted. Despite dramatic success in reducing the number of people who have leprosy, two problems that existed in 1965 persist today. One is the inability to grow the leprosy bacterium in the laboratory. The other is that the neurological complications of leprosy are resistant to therapy. The bacterium causes nerve damage in the peripheral nervous system where it infects the myelin sheath and disrupts nerve functions. (The myelin sheath is the fatty coating that surrounds and insulates nerve cell axons; it is necessary for rapid nerve impulse conduction.) Also, leprosy patients vary widely in their immune responses to infection with *M. leprae*, a phenomenon that is not well understood.

An important scientific contribution of the U.S.–Japan Program was the use of the drug rifampicin to treat leprosy, an advance that can be attributed to U.S. scientists, said Dr. Shimao. Japanese scientists are advancing the development of drug therapy by conducting experiments with animal models.

"The annual panel meeting of the USJCMSP Tuberculosis and Leprosy Panels is *the* premier meeting for the study of leprosy," said Ms. Gail Jacobs, U.S. Secretariat for the Tuberculosis and Leprosy Panel, in a September 2004 interview. "There have been new directions in leprosy research. For example, investigators are applying molecular techniques for the genomic analysis of leprosy isolates, which is really a first in the field."

Today, Japanese and U.S. members of the Tuberculosis and Leprosy Panels freely exchange leprosy research materials, funded in part by an NIH contract. The research materials include polyclonal antibodies raised in rabbits, whole bacterial cells (both irradiated and non-irradiated), whole-cell lysates, subcellular fractions, lipids, and genomic DNA extracted from *M. leprae*. Because it is impossible to grow *M. leprae* in culture, researchers grow the bacteria in live animals and then purify the bacteria from tissues.

Tuberculosis. In 1965, the USJCMSP Panels on Tuberculosis decided to focus their efforts on the immunology and pathogenesis of tuberculosis. Tuberculosis is caused by a bacillus, *Mycobacterium tuberculosis*, which, in 1965, caused active disease in an estimated 12 million people. Today, the tuberculosis bacterium infects an estimated one-third of the world's population, causes active disease in more

than 8 million, and kills more than 2 million people each year. In 2001, Japan reported 35,489 new cases of tuberculosis, of which 11,408 were sputum-positive.

The worldwide problem of tuberculosis has been made significantly worse by the spread of AIDS and the emergence of drug-resistant forms of the tuberculosis bacterium. Many people who are infected with *M. tuberculosis* are co-infected with human immunodeficiency virus (HIV), which has created a pandemic of TB/HIV. In African countries, an estimated 14 million people are co-infected with the tuberculosis bacterium and the AIDS virus. The emergence of drug-resistant forms of tuberculosis in many countries further complicates efforts to control tuberculosis. The number of "hot spots" of multiple drug-resistant strains of *M. tuberculosis* is growing, and now includes Eastern Europe, parts of Latin America, Africa, Asia, and many Pacific countries (Bangladesh, Cambodia, China, India, Indonesia, Myanmar, Pakistan, Philippines, Thailand, Viet Nam, and DPR Korea).

One of the most important advances in tuberculosis research to emerge from the U.S.–Japan Program was the discovery by Japanese scientists in the 1980s that DNA from mycobacteria acts as a strong immunostimulatory agent. Since the 1950s, scientists had recognized that the live, attenuated tuberculosis vaccine had important immunostimulatory properties. This vaccine consists of the Bacillus Calmette-Guerin (BCG), a strain of *Mycobacterium bovis.* The BCG vaccine is widely used around the world (although not in the United States) for preventing tuberculosis in children. It is used for the treatment of bladder cancer in the United States. So it was clear that BCG and other mycobacteria are capable of stimulating macrophage and antitumor activity in animals and humans. However, researchers were not able to identify the mycobacterial molecule that triggered these immune responses. Japanese scientists affiliated with the USJCMSP discovered that DNA purified from BCG was responsible for the activity.[2] It took a long time for this unique idea to be accepted until Drs. Tohru Tokunaga, S. Yamamoto, and their colleagues demonstrated that unmethylated palindromic DNA fragments from bacteria can augment the activity of natural killer cells.[3] The immunostimulatory activities of these DNA oligonucleotides depend on specific base sequences that have CpG motifs. In the 1990s, other scientists showed that bacterial DNA containing CpG motifs could activate mouse B cells. The study of immunostimulatory DNA from bacteria has expanded rapidly, and has led to the realization that the immune responses of higher animals against immunostimulatory DNA might be a primitive and essential way to distinguish self from non-self, thereby nonspecifically fighting bacterial infections. Thus, the work by Dr. Tokunaga and his colleagues can be regarded as one of the most original and important works carried out under the support of USJCMSP Japanese Tuberculosis Panel.

Scientists affiliated with the USJCMSP have also made significant scientific advances in understanding the biology of the mycobacteria that cause leprosy and tuberculosis. The genomes of both *M. leprae* and *M. tuberculosis* have been fully sequenced, an effort that involved scientists in the U.S.–Japan Program who participated in the sequencing of several strains of *M. tuberculosis* and *M. bovis*/BCG, *M. avium*, other mycobacteria, and related actinomycetes. These and other studies make it possible to compare and analyze the genomes of these pathogens, advances that reveal critical information about the biological properties of these organisms and are leading to important discoveries of the molecular basis of bacterial pathogenesis and physiology.

Also, genomic analyses have allowed scientists to advance their understanding of many aspects of the bacteria that cause tuberculosis. Researchers can now: identify the role of certain lipids in disease persistence, and the role of two-component systems in bacteriostasis and perhaps latency; recognize a new, highly immunogenic *M. tuberculosis*-specific protein, which has become the basis of the first subunit vaccine against tuberculosis; apply the use of DNA microarrays to define whole-organism responses to antibiotics and the anoxic conditions that are relevant to latent tuberculosis; identify analogous regions of variation in *M. tuberculosis* genomes, which helps explain its variable virulence and provides a new sets of tools for studying the molecular epidemiology of tuberculosis; and recognize such proteins as ESAT-6 and CFP-10 in *M. tuberculosis* but not in *M. bovis*/BCG, which provides specific antigens for the early diagnosis of tuberculosis.

More USJCMSP-related science advances are on the horizon. A Japanese company has recently developed a new drug for tuberculosis that has no relationship to previous drugs. "It is hoped this new drug will shorten the duration of chemotherapy to 3 to 4 months, or will be able to treat multidrug-resistant tuberculosis," says Dr. Shimao. The drug, which is still unnamed, is now in a phase I clinical trial in the United Kingdom; phase II and III trials are expected to begin in 2005.

The following is a list of other important science advances in leprosy and tuberculosis research to which USJCMSP scientists have contributed. The list is adapted from information published in the USJCMSP five-year reports, as well as information supplied by Dr. Patrick Brennan (member of the U.S. Leprosy Panel from 1983 to 1986, Leprosy Panel Chair from 1986 to 1996, and Co-Chair of the U.S. Tuberculosis and Leprosy Panel from 1996 to 1999), Dr. Masao Mitsuyama (Japanese Panel Chair from 2000 to the present), Dr. David McMurray (U.S. Panel member from 1990–2003, and U.S. Panel Chair from 1999 to the present), and Ms. Gail Jacobs (Secretariat for the U.S. Tuberculosis and Leprosy Panel).

Tuberculosis and Leprosy Panels

[Established as separate Leprosy Panels and Tuberculosis Panels in 1965; amalgamated and renamed in 1996]

[Science Advances: 1965–2000]

Leprosy Advances.

- Made major contributions to the dramatic decline in the number of cases of leprosy. USJCMSP scientists contributed to these advances through epidemiological studies, by conducting initial tests of the mouse footpad/challenge assay for propagating *M. leprae*, and by designing the current multiple drug therapy (MDT) drug regimen for treating leprosy.
- Developed nude mice as a model system for the in vivo growth of *M. leprae*. In 1960, a U.S. scientist (Dr. Charles Shepard, who was Chair of the U.S. Leprosy Panel from 1965–1977) and his colleagues reported the inoculation of human leprosy bacilli into mouse footpad and subsequent multiplication of the bacteria. However, far more significant bacterial growth was obtained by inoculating *M. leprae* into athymic nude mice, a technique developed independently by USJCMSP-supported Japanese scientists in 1976.[4] In the same year, Drs. M. Jo Colston and G.R.F. Hilson reported the same model.[5] Now it is established that the armadillo is the most appropriate animal from which to obtain a large amount of bacilli but, because of their availability and convenience, nude mice are still used as an *in vivo* model system for growing *M. leprae*.
- Developed innovative approaches such as the controlled use of thalidomide to test drugs that could alleviate nerve damage and reversal reactions associated with leprosy.
- Contributed to the sequencing of the *M. leprae* genome in 1998, and the revelation that the complete coding capacity of the organism was contained in a mere 1,600 genes and that one-half of the genome was incapacitated, which accounts for the obligate parasitic nature of the leprosy bacillus. These developments have now allowed the recombinant expression of the 1,600 genes of *M. leprae*, especially those that are unique to the leprosy bacillus, which are proving effective for diagnosing leprosy and defining the molecular basis of disease pathogenesis.
- Characterized the major cell-mediated (Th1 and Th2) immune responses in leprosy patients at different points in the disease process. In patients with tuberculoid leprosy, CD4+ cells and type 1 cytokines (interleukin-2 and interferon-gamma) predominate, and the growth of the mycobacterium is restricted. In patients with lepromatous leprosy, CD8+ cells and type 2 cytokines (interleukins 4 and 10) predominate, and the infection is not controlled. Factors that tip the balance between Th1 and Th2 responses, and immunoprotection versus immunopathogenesis include interleukin-12, as well as the dominance of "special" T cells that bear receptors and recognize antigens that promote cell-mediated immunity.[6]
- Determining the full sequence of the genome of *M. leprae* has resulted in the recognition of nucleotide repeat segments that show variability when compared to the *M. leprae* DNA obtained from patient biopsies across the globe. The recognition of variable numbers of tandem repeats in *M. leprae* genomes has allowed the molecular tracking of isolates and infections in communities, which provides clues about the epidemiology of leprosy and drug-resistant leprosy, a truer estimate of the incidence of global leprosy, and the origins of the undiminished number of new cases observed over the past 10 years. Japanese researchers from the Leprosy Research Center were pioneers in these developments, followed by researchers in the United States.

Tuberculosis Advances.

- Discovery that DNA from tuberculosis mycobacterium acts as a strong immunostimulatory agent. [See discussion above.]
- Discovery of interleukin-5 (IL-5). In the early 1970's, the presence of so-called helper T cells was recognized but the molecules responsible for helper action had not yet been identified. Japanese scientists led by Dr. Kiyoshi Takatsu found a factor in the culture supernatant of PPD-stimulated lymphocytes from mice immunized with *M. tuberculosis* that supported B cell differentiation. They named this factor as TRF (T cell-replacing factor) and carried out an extensive series of studies to clarify the molecular basis of TRF activity.[7] By establishing T cell hybridomas that produce TRF, they succeeded in cloning cDNA for TRF.[8] The recombinant TRF exhibited various interesting biological functions and was named interleukin-5.[9] Now IL-5 is known as a central cytokine that regulates both innate and acquired immunity in the host, and USJCMSP scientist Dr. Kiyoshi Takatsu is known as the discoverer of IL-5.
- Developed and demonstrated the efficacy of the rifamycins for treating tuberculosis and leprosy, by use of increasingly sophisticated clinical trial methods that have resulted in short-course (6-month) treatment regimens for both diseases. USJCMSP scientists assisted the World Health Organization in implementing tuberculosis-control programs, DOTS[10] and DOTS-Plus, on a global scale. The treatment involves an easily followed, short-course (6-month) therapy, thereby ensuring the full course of treatment and preventing the development of multidrug-resistant tuberculosis.
- Characterized the genetic basis in *M. tuberculosis* for resistance to drugs such as isoniazid and rifampin, which has made it possible to track resistant isolates through genetic means and to institute appropriate containment measures
- Elucidated the structure and biochemistry of the mycobacterium cell wall. These studies have enhanced the understanding of how frontline tuberculosis drugs work, helped improve the performance of the drugs, and led to the identification of potential new drug targets for treating tuberculosis. This latest development has invigorated tuberculosis drug-discovery programs, including tests by large pharmaceutical firms to determine the essential molecular targets of *M. tuberculosis*, the development of high-throughput screening processes, and other advances in medicinal chemistry.
- Generated and used animal models (e.g., mouse, guinea pig, and rabbit) to screen dozens of potential vaccine candidates, and elucidate mechanisms of host immune response to *M. tuberculosis* infection.[11] These developments have led to the progression of promising vaccine candidates—through the mouse, guinea pig, and primate models of tuberculosis—such that new subunit and recombinant BCG vaccines are now in phase I human trials.

[Science Advances: 2000–2004]

- Discovered the first evidence of short-tandem repeat (STR-based) polymorphism in *M. leprae*. Until recently, the *M. leprae* genome showed little evidence of polymorphism due, in part, to the loss of insertion sequences and no transposon-mediated polymorphism. However, Dr. Masanori Matsuoka, at the Yokohama meeting in 2000, reported polymorphisms in a hexanucleotide short-tandem repeat (STR) in the *rpoT* gene of *M. leprae*; some isolates had three copies; more had four. Subsequently, he examined isolates from biopsy specimens across the globe and matched the two forms of leprosy to human migration patterns. This first evidence of STR-based polymorphism in *M. leprae* has opened up the floodgates, and extensive collaborative, coordinated studies are underway on the molecular epidemiology of leprosy, based on mini- and micro-satellite STRs and SNPs. This is a major development since the tools are now available at last to trace leprosy transmission patterns.
- Developed new tools for molecular fingerprinting and early diagnosis of *M. leprae* with the promise of better understanding leprosy transmission patterns. The *M. leprae* genome contains only about 1,600 intact genes, more than 1,000 pseudogenes, and has a coding capacity of less than 50 percent. Thus, the genome of the leprosy bacterium has undergone dramatic reductive evolution, and the organism is compromised, particularly in catabolic pathways. This helps explain why past efforts at *in vitro* growth could not have succeeded. USJCMSP scientists have collaborated with other colleagues to cross-reference *M. leprae* genes with those of other organisms, which has led to a core set of 29 putative *M. leprae*-specific proteins that have been generated as recombinant proteins. Potential T-cell reactive peptides from these have also been synthesized. The application of some of these proteins and peptides is making it possible to better understand the patterns of transmission of *M. leprae* among leprosy patients, their household contacts, and other populations.
- Demonstrated, in Japan, a relatively high level of resistance of *M. leprae* to fluoroquinolone (FQ), which is traceable to a point mutation in the *gyrA* gene. Isolates from relapsed Japanese leprosy cases harbored dapsone, rifampin, and quinolone resistance; the percentage of dapsone resistance in cases from the Philippines and Indonesia was as high as 11 percent, and some of these were also resistant to rifampin. The work coincides with other studies at the National Hansen's Disease Center (Baton Rouge, LA) and the Leprosy Research Center, Tokyo, which defined the genetic basis of resistance of *M. leprae* to rifampin and dapsone. Reverse transcriptase-polymerase chain reaction (RT-PCR) technology was used to detect these examples of drug resistance in *M. leprae*. This is an important development, because the universally applied MDT for treating leprosy consists only of rifampin, dapsone, and clofazimine.

- Defined host and immune response in tuberculosis. From 1999 to 2001, Japanese Panel members and participants (e.g., Drs. Masao Mitsuyama, Kiyoshi Takatsu, Yasunobu Yoshikai, Kiyoko Akagawa, Yoshitaka Goto, and Isamu Sugawara) made major progress in defining the host response to tuberculosis. For example, they demonstrated that escape from the phagosome compartment into the cytosol is essential in cytokine expression; defined the peptide epitope in the major α-antigen responsible for induction of $CD4^+$ TH1 cells; and described the differential roles of GM-CSF and M-CSF in the ability of human-derived macrophages to handle virulent *M. tuberculosis*.
- Helped define the roles of various cytokines, chemokines, and T- and B-cell subsets in disease resistance and progression using knockout mice. Dr. Sugawara demonstrated a selective role of MyD88 in granulomatous formation, which is independent of susceptibility to infection. By using the large array of knockout mice variants, the roles of almost all known cytokines and chemokines in tuberculosis development have been defined. For example, the role of TNFα has been defined in this way and also with the use of TNFα antibodies. The work of Drs. Flynn and Kaplan has shown that TNF depletion results in greater histopathology, higher bacterial load, and greater mortality. Not only have the roles of TNFα and IFNγ, but also the involvement of IL-12, IL-10, IL-15, IL-18, granulysin, the CAP proteins, etc., in tuberculosis have been largely defined by the combined work of both panels.
- Discovered the role of TLR (Toll-Like Receptors) in the early immune response. For instance, Dr. Fenton reported that both TLR2 and TLR4, activated by LAM (lipoarabinomannan) of M. tuberculosis, were mediators of macrophage activation, NF-kappa B activation, and TNFα production; however, TLR4 was not involved in nitric oxide production. It is now likely that the full range of host-cell receptors and bacterial ligands involved in the initial interaction between M. tuberculosis and macrophage has been defined.
- Improved understanding of the cell biology of the intracellular fate of *M. tuberculosis*. Dr. Anne Goldfeld and her colleagues are implicating new, as yet undefined, genetic factors that control the cellular immune response to *M. tuberculosis*. The basic observation was that PPD non-reactive patients with tuberculosis produced IL-10 but not IFNγ. In contrast, PPD-reactive patients produced both. The IL-10-only producing cells had defective phosphorylation of the TCR chains and defective activation of IAP-70 and MAPK. Members of the Japanese and U.S. Panels are investigating the roles of the CDI *versus* the MHC pathways in acquired protective immunity and nature of the bacterial ligands.
- Made progress toward a tuberculosis vaccine based on a recombinant BCG strain that over-expresses the antigen 85 (Dr. Marcus Horwitz) and a subunit fusion protein, so-called 72f (Dr. S. Reed). The vaccine is now in Phase I clinical trials. The MPL (monophosphoryl lipid A) adjuvant, used with many promising vaccines today, has been approved for human use and is a product of the combined genius of early U.S.–Japan Panel members, Drs. Ichiro Azuma and Edgar Ribi.
- Made progress toward the development of new, anti-tuberculosis drugs. USJCMSP scientists defined the mode of action and genetic/biochemical basis of drug resistance of pyrazinamide a pro-drug that is activated to pryazinoic acid by the *pyrA* gene product, the site of Pyr resistance. Drs. Hatsumi Taniguchi and Y. Suzuki have defined the resistance mechanism to kanamycin (KM). Dr. Patrick Brennan resurrected a clinically acceptable anti-tuberculosis drug from the 1960s, a thiourea isoxyl, demonstrating that it had a completely unique mode of action. Several new categories of drugs have been identified and are in various stages of testing by both Japanese and U.S. investigators: the oxazolidinones, the nitroamidzopyrans, new rifamycins, fluoroquinolones, and ethambutol derivatives. These drug development studies have been greatly facilitated by development in high-throughput screening (HTS), such as proximity assays, incorporation of luciferase into test strains of *M. tuberculosis*, the use of molecular beacons and radiorespirometry.
- Made advances in the diagnosis of tuberculosis, which have benefited from refinements in DNA amplification to detect M. tuberculosis and drug-resistant forms. Commercial nucleic acid kits have now been approved for application to smear-positive and smear-negative tuberculosis; both luciferase expression and molecular beacons are being applied to detection of rifampin resistance. The commercial Quantiferon® that was originally developed for bovine tuberculosis based on bovine PPD and allowing the ready detection of γIFN in whole blood PBMCs, is now being applied to the diagnosis of human tuberculosis, but based on M. tuberculosis-selective antigens, such as CFP10 or ESAT-6.

Footnotes — Tuberculosis and Leprosy Panels

[1] According to data tabulated by the World Health Organization (WHO) in 2002, six countries—Brazil, India, Madagascar, Mozambique, Myanmar, and Nepal account for 90 percent of the world's leprosy cases, and 70 percent of the cases occur in India. [From WHO Media Center, Fact Sheet No. 101: Leprosy. http://www.who.int/mediacentre/factsheets/fs101/en/]

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[10] "The internationally-recommended TB control strategy is DOTS. DOTS combines five elements: political commitment, microscopy services, drug supplies, surveillance and monitoring systems, and use of highly efficacious regimes with direct observation of treatment." [From WHO Tuberculosis Strategy & Operations, Monitoring & Evaluation: What is DOTS? http://www.who.int/gtb/dots/whatisdots.htm]

[11] 35 Years of Progress. U.S.–Japan Cooperative Medical Science Program. Seventh Five Year Report: 1996–2000.