Focus on Tuberculosis

By Ratnakar P. Kini

The contents of this course are taken from the National Institute for Allergy and Infectious Diseases, NIH. Learning objectives and post test have been preparedby Dr. Ratnakar P. Kini.

Objectives

Upon completion of this course, the learner will be able to:

1) Explain how tuberculosis is caused?
2) Discuss about the treatment and vaccinations
3) Explain how the disease has emerged as global problem?
4) Discuss about the recent trends in the diagnosis and treatment of tuberculosis
5) Explain about the various clinical trials conducted all over the world on tuberculosis?

Introduction: A Terrible Toll

Tuberculosis (TB) is an old disease but one that still ranks among the foremost killers of the 21st century. Every second of every day, someone is newly infected with the bacterium, *Mycobacterium tuberculosis* (*M. tb*), that causes TB. About one-third of the world's population is infected with *M. tb* , and as many as two million people die of the disease each year. TB kills more people than any other disease caused by a single infectious agent. Among people with HIV/AIDS, tuberculosis is the leading cause of death. The highest rates of TB are in some of the world's poorest countries, and the economic toll taken by the disease is enormous. Left unchecked, infectious disease can sow the seeds of political upheaval and threatens to reverse progress made by developing countries in recent decades. As for the toll in lives shortened, children orphaned, and communities weakened, the cost is inestimable.

Unprecedented Opportunities

Among the components of the National Institutes of Health (NIH), the National Institute of Allergy and Infectious Diseases (NIAID) is the primary locus of TB research. NIAID has been committed to fighting infectious disease and improving global health throughout its history. From state-of-the art laboratories in the United States to busy clinics in South Africa, scientists supported by NIAID are learning new things about the TB bacterium and developing better ways to fight the diseases.

“Better control and eventual elimination of TB worldwide will require a marriage of modern science, time-tested public health measures, and the strong commitment of the international community," says NIAID Director Anthony S. Fauci, M.D. “History will judge us harshly if we do not capitalize on unprecedented opportunities and act boldly to rid the world of this ancient killer.”

TB is a worldwide problem
Credit: Clifton E. Barry, III, Ph.D.

Tuberculosis in History

"I Must Die"

Tuberculosis, it seems, has always been with us. Evidence of tubercular decay has been found in the spines of Egyptian mummies thousands of years old, and the disease was common both in ancient Greece and Imperial Rome. While it may have lessened its grip on mankind during some periods of history, TB never completely let go.

The Poet John Keats
Credit: Unknown

Attempts at cures were varied, but uniformly ineffective. Roman physicians recommended bathing in human urine, eating wolf livers, and drinking elephant blood. Fresh milk—human, goat, or camel—figured in many treatment regimens. Depending upon the time and country in which they lived, patients were exhorted to rest or to exercise, to eat or to abstain from food, to travel to the mountains or to live underground.

And yet, tuberculosis continued to claim victims by the millions. When, in 1820, the poet John Keats (who had schooling in medicine) coughed a spot of bright red blood, he told a friend, "It is arterial blood. I cannot be deceived. That drop of blood is my death warrant. I must die." Within a year, at just 25, he did.

Consumed by Love

Other artists and writers who succumbed to tuberculosis in the 19th century included Frederick Chopin, Anton Chekov, Robert Louis Stevenson, and Emily Bronte, while 20th century victims included Franz Kafka, George Orwell, and D.H. Lawrence. Consumption became romanticized in the popular imagination as a disease of the young, pure, and passionate. The heroines of Alexandre Dumas' 1852 novel, Camille, and Giacomo Puccini's 1896 opera, La Boheme, were among the fictional characters whose deaths from tuberculosis were imagined to result from thwarted love affairs.

Poster advertising Puccini's opera
Credit: Unknown

A Doctor's View

The variable course of TB only served to make it more baffling and terrifying. Physicians could not easily predict whether a consumptive patient would succumb within months, linger for years, or somehow manage to overcome the disease altogether. According to the 19th century American physician William Sweetser, the first stage of consumption was marked by a dry, persistent cough, pains in the chest, and some difficulty breathing, any of which could be symptoms of less dire illnesses.

The second stage brought a cough described by Dr. Sweetser as "severe, frequent, and harassing" as well as a twice-daily "hectic fever," an accelerated pulse, and a deceptively healthy ruddiness in the complexion.

In the final, fatal stage, wrote the doctor, "the emaciation is frightful and the most mournful change is witnessed...the cheeks are hollow…rendering the expression harsh and painful. The eyes are commonly sunken in their sockets...and often look morbidly bright and staring." At this point, throat ulcers made eating difficult and speech was limited to a hoarse whisper. Once the distinctive "graveyard cough" began, diagnosis was certain and death inevitable. Rarely, wrote Dr. Sweetser, "life, wasted to the most feeble spark, goes out almost insensibly." More typically, severe stomach cramps, excessive sweating, a choking sensation and vomiting of blood preceded the victim's demise.

A New Theory

As long as the cause of tuberculosis remained unknown, efforts to cure it were based more on trial and error than on any scientific reasoning. In general, consumption was not thought of as a contagious disease. Rather, most believed it to be hereditary, and a result, at least in part, of an individual's mental and moral weaknesses. The English physician Benjamin Marten was among the first to propose an alternative in his 1720 publication, A New Theory of Consumption. Marten believed that "wonderfully minute living creatures," which could be spread by prolonged close contact between infected and healthy individuals, caused tuberculosis.

His conjecture would remain just that for another century and a half. Then, on an extraordinary evening in March 1882, an obscure German country doctor astounded the European medical establishment and became an overnight sensation with his proof that the dread disease that had felled so many was caused by a microscopic organism. The doctor was Robert Koch and the announcement opened the way to an age of optimism in the battle against TB.

Age of Optimism

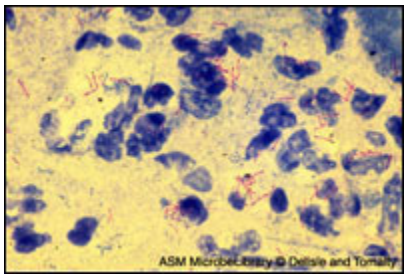

German microbiologist Robert Koch

On the evening of March 24, 1882, before a skeptical audience of Germany's most prominent men of medicine, Robert Koch announced a discovery that awed his listeners and brought him worldwide acclaim. New laboratory techniques had helped scientists discover microbial causes of other infectious diseases, but tuberculosis remained a stubborn exception. Koch succeeded by staining the organism with not one but two dyes, and at last was able to bring the elusive microbe into view. "Under the microscope the structures of the animal tissues, such as the nucleus and its breakdown products are brown, while the tubercle bacteria are a beautiful blue," he wrote in the paper that followed his dramatic presentation that March evening.

Koch was the first to get colonies of TB bacteria to grow in the lab. The bacteria, he discovered, are extremely slow-growing, requiring two weeks before clumps of them could be seen with the naked eye. Further research by Koch revealed that TB was spread from person to person and, as such, potentially controllable. Also, the way TB spreads—carried on droplets expelled in an infected person's cough—became clear. An optimistic Koch wrote, "When the conviction that tuberculosis is an exquisite infectious disease has become firmly established among physicians, the question of an adequate campaign against tuberculosis will certainly come under discussion and it will develop by itself."

Resting the Lungs

A key element in that campaign was a public health movement that isolated the sick—sometimes by force—from the well. This era of TB sanatoria actually began in 1849, well before the scientific proof of TB's contagious nature. A consumptive German doctor traveled to the Himalayas and returned cured. The doctor, Hermann Brehmer, became convinced that life at high elevation, continuous exposure to fresh air, sun and cold, along with copious amounts of food, could turn TB from a death sentence into a curable disease. In the United States, less emphasis was placed on high elevation, but the basic outlines of sanatorium care and philosophy remained the same.



Magnified image of TB bacteria
Credit: ASM MicrobeLibrary, Electron micrograph by Delisle and Tomally

A young doctor named Edward Livingston Trudeau established the most famous sanatorium in the United States at Saranac Lake, in New York's Adirondak Mountains. Like Brehmer, Trudeau suffered from TB and was informed by his doctors that he would not live long. In 1882, Trudeau became aware of Koch's experiments with TB bacteria and of Brehmer's sanatorium. Although very weakened by his illness, Trudeau established a small laboratory in Saranac Lake and began an extraordinary series of experiments.

Edward L. Trudeau in his laboratory
Credit: The Adirondack Museum, NY

Five rabbits were inoculated with TB bacteria and set loose on a small island where they were provided with fresh vegetables in addition to the naturally occurring grasses. After four months, one rabbit died, but the others remained robustly healthy. Upon autopsy of the apparently healthy rabbits, no evidence could be found of the point of inoculation, reported Trudeau in an 1887 paper. Evidently, the rabbits' healthy life permitted them to fend off infection.

Believing his findings gave a strong empirical basis to the European-style sanatorium treatment regimens, Trudeau instituted many of those regimens in the "cure cottages" he established at Saranac Lake. Patients were under strict and constant supervision; every aspect of their lives was detailed in rule books issued to each invalid. Typically, a newcomer spent a minimum of three months on complete bedrest. The resident was exposed to fresh air for most of the day and was required to consume enormous amounts of food, including many servings of milk each day.

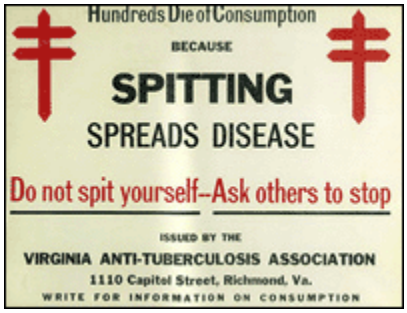
Public Health and a New Vaccine

Patients in a Canadian sanatorium
Credit: Ninnette TB sanatorium, Manitoba, Canada—Courtesy of Ian Carr

A noticeable decline in the incidence of TB in the United States began around the turn of the century. Some of the decline was probably due to a natural waning of the epidemic, but some was also the product of quarantining the sick in sanatoria and of aggressive public health education campaigns. Publications aimed at children and adults admonished against spitting and recommended plenty of sleep, fresh air and exercise for everyone.

In 1908, French scientists Albert Calmette and Camille Guerin developed a vaccine against TB. They began by isolating *Mycobacterium bovis* (which causes TB in cattle) from a dead cow. Every three weeks for the next 13 years, the scientists grew a new batch of bacteria in a solution of beef bile and potatoes. Each new generation of bacteria was weaker than the one before. Eventually the bacteria lost the power to cause disease, but could still provoke the immune system to protect a person from TB. The vaccine Bacille Calmette-Guerin (BCG) was first administered in 1921 to an infant whose mother had died of TB. Since then, more than a billion people have been inoculated with the cheap and safe BCG vaccine. The vaccine's efficacy, however, is unclear. The consensus is that while BCG vaccine can prevent TB infection in the brain among children, it is nearly useless in preventing adult pulmonary TB.

Drugs Vanquish TB



A public health campaign poster
Credit: Historical Collections and Services, The Claude Moore Health Sciences Library, University of Virginia

During the 1940s, TB vaccines took a back seat to advances in drug therapy. Dogged effort by the American scientist Selman Waksman produced streptomycin, a relatively non-toxic antibiotic derived from a soil fungus. On November 20, 1944, a critically ill TB patient received streptomycin. Within days, he began a near-miraculous recovery. A host of drugs followed on the heels of streptomycin and, when used in combination, they could usually cure TB without engendering drug-resistant bacteria. Some of the most important drugs introduced during the 1940s and '50s included isoniazid, rifampin, and ethambutol.

Effective drugs brought the sanatorium era to a close. Some research journals devoted to tuberculosis ceased publication, and organizations such as the American Lung Association (formerly known as The National Association for the Study and Prevention of Tuberculosis) redirected their efforts. Confidence arose that TB, like infectious diseases of previous centuries, could be completely conquered by drug therapy. Indeed, the United Nations predicted the

worldwide elimination of TB by the year 2025. But this ancient enemy was not so easily routed.

A Killer Returns: The Face of the Epidemic

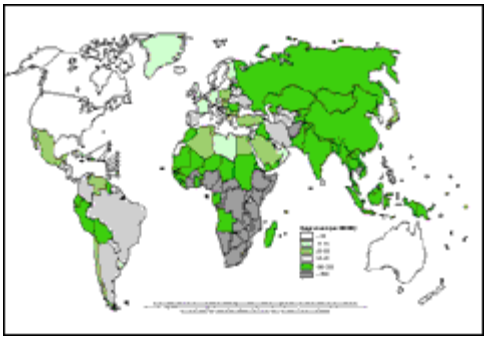
Tuberculosis continues to exact its terrible toll on humankind. Worldwide, a person is newly infected with TB every second, and overall nearly two billion people have been exposed to TB bacterium. During the 1990s, bright hopes that the disease would be vanquished by 2025 were extinguished as a variety of medical and social factors helped TB surge back to its familiar position among major causes of death.

Around 1985, cases of TB began to rise in the United States. Several interrelated forces drove the resurgence, including increases in prison populations, homelessness, injection drug use, crowded housing, and increases in populations of long-term care facilities. Along with increased immigration of people from countries where TB is endemic, these forces provided ideal conditions for TB transmission. Adding the most fuel to the fire, however, were the HIV/AIDS epidemic and increases in multidrug-resistant TB (MDR-TB).

TB strikes hardest in young adulthood Credit: World Health Organization

A Global Emergency

TB is a contagious disease. When people with active TB cough, spit, or even talk, bacteria that cause the disease are propelled into the air. A person needs to breathe in just a few TB bacteria to become infected. Without treatment, a person with an active case of TB will infect between 10 and 15 people a year. Infection with TB bacteria, however does not necessarily lead to disease. In a person with a healthy immune system, TB germs take up residence in lung cells, but enter a kind of suspended animation and never cause widespread disease. Only between 5 and 10 percent of all healthy people infected with the germ will develop active TB at some point. In persons with decreased immune function, whether due to HIV/AIDS infection, poor nutrition, or old age, the odds are much worse. When infected with both HIV and TB, for example, a person has a one in ten chance of developing active TB each year (compared with a one in ten chance over a lifetime for people without HIV).



A worldwide epidemic
Credit: World Health Organization

TB kills between 2 and 3 million people each year, and is the leading cause of death among young adults and a major cause of death among women of childbearing age. So great was the concern about the worldwide epidemic of TB that in 1993, the World Health Organization (WHO) declared tuberculosis a global emergency, the first time a disease had ever achieved that dubious distinction.

Birth of a Superbug

Perhaps the most alarming aspect of the present epidemic is the rise in multidrug-resistant TB (MDR-TB). According to a survey conducted by the WHO, up to four percent of all TB cases worldwide are resistant to more than one anti-tuberculosis drug. In parts of Eastern Europe, nearly half of all TB cases resist at least one first-line drug. Most of the burden of MDR-TB falls on poor countries, but the United States has seen outbreaks of drug-resistant TB as well. In early 1990s, New York City had an epidemic of MDR-TB that cost almost $1 billion to control.

With proper treatment, almost all cases of TB are curable. But proper treatment is not always easy to attain. Typically, a TB patient takes four different antibiotics for at least two months, then two drugs for four more months. Hitting TB germs with several drugs simultaneously lessens the chance that naturally occurring mutations in the bacteria will allow some to escape destruction. However, because the drugs often cause unpleasant side effects and because patients start feeling better after month or so, not everyone completes the full course of treatment. In many less developed countries, where TB is most common, drug supplies may be inadequate and medical services spotty.

Unfortunately, partial treatment for TB is worse than no treatment at all. TB bacteria that linger following incomplete therapy are likely to resist anti-tuberculosis drugs in future flare-ups. Worse still, people with active cases of MDR-TB can pass those superbugs on to new victims.

Understanding TB

The Immune Response to TB

"In my research," says Gilla Kaplan, Ph.D., of the Public Health Research Institute in Newark, New Jersey,


Gilla Kaplan, Ph.D.

"I ask what aspect of the immune response to infection protects some people from developing TB, and what is missing in those people who develop the disease."

Both human and bacterial factors contribute to the eventual outcome of *Mycobacterium tuberculosis (M. tb)* infection. If scientists could identify how these host and pathogen factors interact, there might be ways to, for example, boost immune responses or draw the TB bacteria out of latency and make them more vulnerable to drug attack.

One response under study by Dr. Kaplan is inflammation caused when a chemical called TNF-α is released from certain immune system cells exposed to *M*. tb. Some inflammation is good because it helps the body eliminate the disease-causing organisms; but uncontrolled inflammation can cause just as much damage as the disease itself. Dr. Kaplan and her colleagues are studying whether the drug thalidomide and its analogues can dampen excess inflammation caused by TNF-α. Although the approach has not yet been tested in humans, it has shown promise in animal models of TB.

Dr. Kaplan's lab also looks at the problem from the bacterium's viewpoint. Human immune system weapons deployed early—just after the bacterium invades the lung—differ from those used by the immune system during latent infection, Dr. Kaplan explains. To survive in humans, TB bugs must switch on different genes in response to the changing immune response. In effect, the bacteria's changing genetic profile mirrors the human immune responses, Dr. Kaplan says.


Micrograph of a human macrophage extending pseudopods to embrace bacteria
Credit: Bayer Laboratories

She and her collaborators, including Dr. McKinney of Rockefeller University, sought a better understanding of this molecular mirror by analyzing lung tissue samples taken from two groups of TB patients. The first group had active TB; the second group of patients was infected, but did not have any TB symptoms. Dr. Kaplan's team is pinpointing which human immune response genes are expressed in these various states, while researchers in Dr. McKinney's lab are determining which bacterial genes are switched on at each phase. The scientists will

use what they learn from these experiments to improve a rabbit model of TB, which should better mimic latent TB infection in humans.

Late in 2004, Dr. Kaplan collaborated with NIAID researchers in NIAID's Division of Intramural Research, led by Clifton E. Barry, III, Ph.D., to discover how a particularly virulent strain of *M. tb* causes severe disease. Evidence from TB clinics suggested that families of strains of *M. tb* called W-Beijing are more likely to cause severe disease and to be multi-drug resistant than other strains. In mice, members of the Strain W-Beijing are deadly. Drs. Kaplan and Barry found a molecule produced by W-Beijing strains that seems to prevent immune system cells from releasing several infection-fighting chemicals. This molecule, PGL, is not produced by many TB strains.

When the NIAID scientists altered the TB bacteria to remove their ability to produce PGL, the bacteria were not as lethal to mice, although the organisms could still reproduce inside the lung. The research that found a link between PGL production and "hyper-lethality" of certain TB strains was conducted in mice, so any role that PGL may have in human TB must still be determined, notes Dr. Kaplan. She and colleagues in Cape Town, South Africa, and Dr. Barry and colleagues in Masan, South Korea, are now beginning the human studies needed to see if PGL-producing strains cause more severe disease or are more likely to be resistant to TB drugs than TB strains that do not make PGL.

TB Research Attains New Heights in Colorado


Ian Orme, Ph.D.

"This is a jolly interesting time in tuberculosis research," says Ian Orme, Ph.D., of Colorado State University (CSU). Dr. Orme has not always been as optimistic as he is today and, considering how difficult research on tuberculosis can be, it is easy to understand why; the microbe that causes tuberculosis demands careful handling if it is to perform at all.

Roadblocks in Research

Mycobacterium tuberculosis (M. tb) is surprisingly difficult to grow under laboratory conditions and, when it does, lab personnel must be protected from accidental infection. Work on virulent strains of *M. tb* must take place in sophisticated biosafety level three (BSL3) laboratories, of which there a limited number in the United States.

TB research has also been hindered because scientists lacked animal models that could faithfully mimic the disease. For years, investigators studied TB in mice and guinea pigs by injecting the bacterium directly into the animals' bloodstream. Although researchers could learn some things about late-stage tuberculosis with these animal models, the early and more typical pulmonary form of the disease could not be easily studied.

Until recently there was no rapid, accurate way to screen potential drug candidates for their anti-TB properties, creating still another roadblock. Add to this the limited interest large pharmaceutical companies have shown in bringing new anti-TB drugs to market and it's not surprising that veteran TB researchers became disheartened.

Happily, much has changed. Dr. Orme credits NIAID's support for a TB research materials, vaccine, and drug testing facility at CSU with hastening that change. The facility at CSU includes a state-of-the-art BSL3 laboratory where virulent strains of *M. tb* can be grown, stored and distributed upon request to researchers throughout the country.

Animal Models

In the early 1990s, Dr. Orme and his colleagues made an important contribution to speeding TB research when they developed a low-dose aerosol animal model of the disease. Their technique exposes mice to small amounts of *M. tb* delivered in a mist, thus mimicking the usual route to infection.

Dr. Orme uses the low-dose aerosol technique to probe basic questions of how immune system cells in the lung react to *M. tb* during the microbe's initial invasion. The model is playing an important role in applied research on new anti-TB drugs and vaccines too. Using this infection method on knock-out mice (genetically engineered to lack specific genes), Dr. Orme can determine the response an animal is having to a drug candidate in only five days, instead of the previously needed 30 days. He and other research teams can now screen significantly more compounds for possible activity against TB.

Dr. Orme and his colleagues also investigate the behavior of *M. tb* in test-tube experiments. Here, their primary interest is in studying *M. tb* growth when the organism is deprived of oxygen. This may mimic the situation in the lungs of people who have chronic or latent, rather than active, TB. At the moment, there is no good animal model of latent tuberculosis, says Dr. Orme, although he and other research groups are attempting to develop one.

Vaccine Efforts

The expertise and technologies available at the BSL3 site have also spurred research on vaccines. In 1997, NIAID expanded CSU's contract to include a vaccine screening service. Now, researchers from around the world can send candidate vaccines to be tested—at no cost—in the facility. To date, more than 120 vaccine candidates have been assessed for their ability to prevent infection in small animals.

TB bacteria grown in a CSU laboratory
Credit: John Belisle, Colorado State University

On the vaccine development front, Dr. Orme again expresses optimism. Researchers in his lab

are working to identify *M. tb* proteins that elicit a response from the immune system. This follows on the hypothesis, now widely accepted, that proteins secreted by the bacterium after it takes up residence inside the macrophage are targets for immune system action. A better understanding of these bacterial proteins might lead to vaccines designed to produce more of the immune system substances capable of destroying *M. tb.*

According to Dr. Orme, one of the remaining stumbling blocks to even greater progress in tuberculosis research is attracting enough top caliber students into the field. That, too, is getting easier to overcome, thanks in part to facilities for encouraging collaboration, such as those supported by NIAID. Then, too, there is Dr. Orme's own commitment to the cause. He is motivated to keep going by the enormity of worldwide TB. "The global scenario—if we don't improve our ability to treat and prevent this disease—is terrifying," he says.

Of Mice and Monkeys: Animal Models of TB Could Speed TB Treatment

Johns Hopkins University TB researchers, from left, Richard Chaisson, M.D., Jacques Grosset, M.D., William Bishai, M.D., Ph.D., and Eric Nuermberger, M.D.
Credit: Keith Weller

Researchers from The Johns Hopkins University's Center for Tuberculosis Research in Baltimore are tackling one of the toughest problems in TB research—how to shorten the course of drug treatment for the disease. Currently, a complicated regimen of multiple antibiotics must be taken daily for six months or more. The most widely used way to administer TB drug therapy, called directly observed therapy, short-course, or DOTS, is hard and expensive to implement. The cost of the drugs can exceed the cost of hiring qualified observers to carry out the program, for example.

Hopkins researchers Jacques Grosset, M.D., William Bishai M.D., Ph.D., and Richard Chaisson, M.D., focus much of their research on a drug called moxifloxacin, or MXF, which is used to fight other bacterial infections. In 2003, their team, including fellow Hopkins TB researcher Eric Nuermberger, M.D., found that adding MXF to two other anti-TB drugs cut two months off the time normally required to cure mice of experimentally induced TB. The dosage and timing of this mouse TB drug regimen is designed to closely imitate drug activity patterns in human drug regimens. This study provides a strong rationale for considering clinical trials in humans, according to Dr. Grosset.

At the University of Pittsburgh School of Medicine, JoAnne Flynn, Ph.D., and her colleagues are developing a model of TB in non-human primates. Using the cynomolgus macaque, the researchers have created a model of TB that closely mimics both the active and latent stages of TB infection in humans. A non-human primate model of TB enables researchers to access lung tissue samples during latent and active disease, something that is not generally available from human patients, notes Dr. Flynn. Non-human primate models also would aid vaccine designers by providing information about the protective immune responses that fight off TB infection. Finally, this model would allow the study of drugs that might work against latent infection.

The University of Pittsburgh researchers used cynomolgus macaques to study a key process in TB infection, the formation and maintenance of granulomas. Tubercular granulomas are nodules containing *M. tb* -infected immune cells called macrophages. The granulomas keep *M. tb* from spreading by "walling-off" the infected area from the rest of the lung. A host of signaling molecules called chemokines and cytokines are likely employed by the immune system to help form and maintain granulomas, but their respective roles are not yet well understood.

Dr. Flynn and her colleagues, including Dr. Todd Reinhart, examined more than 300 granulomas that developed after the scientists infected monkeys with low doses of *M. tb* . Granuloma formation in macaques closely mimics that seen in humans. The study revealed that one class of chemokines was abundant in the gramulomas. The molecules likely serve to recruit specific kinds of immune cells to the areas where *M. tb* is present, the scientists reported. These findings provide new insight into the way immune system components and the TB bacterium may interact in the human lung.

Diagnosing TB

Shining a Molecular Flashlight on the TB Bacterium


William Jacobs Jr., Ph.D.

William R. Jacobs Jr., Ph.D., has a take-no-prisoners philosophy when it comes to fighting tuberculosis. "Our mission is to eradicate TB and I'll do whatever it takes to get there."

In the campaign against TB, Dr. Jacobs has frequently deployed a miniature army of phages. Phages are viruses that infect only bacteria and are exceedingly common in nature. Dr. Jacobs, who is a Howard Hughes Medical Institute researcher at Albert Einstein College of Medicine in New York City, has isolated phages from Bronx Zoo dirt.

A Miniature Army

In the wild, phages infect their targets by injecting DNA into the bacterial genetic material. This leads to the production of more phages and, eventually, to the death of the bacterium. In the lab, scientists can modify phage DNA with genetic material from other organisms. These genetically engineered phages are like tiny cargo ships that efficiently shuttle the foreign genes into bacteria. So useful is phage technology that Dr. Jacobs says he has used it to surmount every obstacle he has encountered in his research on *Mycobacterium tuberculosis (M. tb)*, the bacterium responsible for TB.

Electron micrograph of a phage
Credit: Robert Duda, University of Pittsburgh

When faced with the problem of developing a rapid, accurate test for determining the antibiotic susceptibility of various strains of *M. tb*, phages once again proved their mettle. This time, Dr. Jacobs and his colleagues developed a phage that carries the gene for luciferase, the chemical that makes fireflies glow. When luciferase-producing phages infect living cultures of *M. tb* and begin to reproduce, the cultures glow. If an antibiotic-sensitive strain of M. *tb* is infected with luciferase phages and subsequently exposed to an antibiotic, the light is extinguished because the phages have no living bacteria to infect. The technique was described in a paper published in 1993.

The Bronx Box

Dr. Jacobs' team built a prototype device, nicknamed the Bronx box, that used inexpensive and sensitive dental X-ray film to detect the glow emitted by phage-infected bacteria.

In 2001, Dr. Jacobs joined with scientists from Stanford University and Mexico's National Institute of Public Health and Nutrition to see if luciferase-reporter phage technology could help clinicians determine to which antibiotics a given strain of TB is susceptible. As Dr. Jacobs notes, drug susceptibility tests are usually not done on clinical samples from TB patients in the developing world; it is simply too expensive and time-consuming. However, incomplete or incorrect drug treatments not only fail to cure the patient, they also lead to ever more prevalent strains of drug-resistant *M. tb*.

Bronx Box—
Sequella, Inc.
Credit: Martyn Green

The Bronx box performed admirably in the Mexican study. The technology proved very accurate both in detecting *M. tb* in samples drawn from Mexican TB patients, and in determining to which antibiotics those isolates were susceptible. The time needed to do these drug susceptibility tests was shortened by more than a week when compared with conventional methods. More studies are underway to evaluate the use of this system in other countries, including India and South Africa.

In 2003, Dr. Jacobs and co-authors from the Centro Internacional de Entrenamiento e Investigaciones Medicas, in Cali, Colombia, published results showing that the low-tech Bronx box and a more sophisticated luciferase-reporter device called a luminometer both performed well in accurately identifying drug resistant strains of TB taken from patients. The luminometer produced a result after only 54 hours, while the Bronx box took 96 hours. Both worked much faster than the "gold standard" test for TB infection, which takes three weeks.

A Timely Test: Real Time PCR

A case of TB that does not respond to two or more of the first-line TB drugs is termed multi-drug-resistant TB (MDR-TB), and it is on the increase in many parts of the world. Patients with MDR-TB are significantly more likely to die of their disease than patients with drug-susceptible strains of *Mycobacterium tuberculosis (M. tb)* and the cost of treating MDR-TB can run into the thousands of dollars per case.

Fast, accurate diagnosis of MDR-TB, particularly in resource-limited settings, is critical if the rise of this especially deadly form of TB is to be slowed, says David Alland, M.D., of The University of Medicine and Dentistry of New Jersey, in Newark. It is important to distinguish patients who have drug-susceptible disease and can be started on a routine regimen of one or more TB drugs from people whose infections are caused by drug-resistant strains of *M. tb.* If the latter can be identified as soon as they come to the clinic for treatment, says Dr. Alland, they can be safely separated from the rest of the hospital patients and will not spread MDR-TB to others.

Dr. Alland and his collaborators at Cepheid, Inc., of Sunnyvale, CA, have had success identifying MDR-TB using a test that they say is well suited to conditions in resource-poor countries where TB is rife. Essentially, explains Dr. Alland, the "molecular beacon assay" is a biotech lab in a test tube. The test quickly, automatically, and accurately tells doctors whether a sample of patient sputum (material expelled from the lungs and throat by coughing) contains drug-resistant or drug-susceptible *M. tb.* The entire test is contained in a sealed cartridge, so the sample cannot become contaminated and give false results.

After the sputum is liquefied and washed, DNA from any *M. tb* present is first extracted and then rapidly expanded through a technique called real-time PCR. When the sample is large enough, five kinds of lab-made fluorescent molecular beacons are applied simultaneously. Each beacon glows in a different color when it attaches to *M. tb* DNA. In the presence of drug-susceptible TB, all five colors are visible. If any one of the colors is absent at the end of the test, it means the sample contains drug-resistant *M. tb.* The initial version of the test detected strains resistant to the TB drug rifampin. In their first publication about this technique, Dr. Alland and his colleagues reported that their molecular beacon assay took less than three hours and correctly identified rifampin-resistant TB in 11 out of 11 sputum samples.

Since then, Dr. Alland and colleagues in the United States, Mexico, and India have improved the assay's accuracy and sensitivity. In 2004, the scientists used the molecular beacon assay to correctly distinguish rifampin-resistant and rifampin-susceptible strains of *M. tb* in patient sputum samples taken from areas of high TB incidence in north India and Mexico.

Larger trials of the assay and sputum processing techniques are being planned in collaboration with Cepheid, Inc., other investigators from University of Medicine and Dentristry of New Jersey, and the Uganda National TB and Leprosy Control Programme, says Dr. Alland.

Blood Evidence: Antibody Tests that Detect Reactivating TB


Maria Gennaro, MD

Controlling TB hinges on distinguishing latent (symptom-free) TB infection from active disease, notes Maria Laura Gennaro, M.D. Current diagnostic tests, she says, are unsatisfactory because they are too slow, inaccurate, or hard to conduct. Moreover, they do not distinguish between different stages of infection with *M. tb.* With her colleagues at the Public Health Research Institute in Newark, New Jersey, Dr. Gennaro is examining blood of *M. tb*-infected people to gain clues to the "evolution of infection."

Simply knowing that a person is infected with *M. tb* is not very meaningful, notes Dr. Gennaro. In poor countries, where the infection rate can be 70 to 80 percent, it would cost too much to treat people who are infected but who will not become sick. Therefore, clinicians focus treatment efforts mostly on people with active TB and on young children who are in close contact with TB patients. Even in wealthy countries, infected people can be reluctant to start a long course of drug treatment for a condition with no symptoms.

To distinguish among latent, active, and reactivating stages of TB, researchers in Dr. Gennaro's lab are determining "profiles" of blood-borne immune system molecules called antibodies. Antibody assortments, or profiles, change over the course of infection

as bacteria themselves shift from one life state to another. If the scientists could discover a correlation between a specific antibody profile and the stage of infection, they could develop powerful tools for distinguishing persons harboring actively growing *M. tb* from people carrying non-growing *M. tb*, explains Dr. Gennaro. Also, she says, a shift in antibody profile might provide an early warning that TB is starting to reactivate. Such an early warning signal would allow doctors to treat the awakening TB before symptoms became obvious.

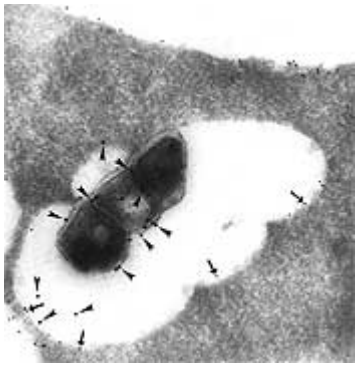
Preventing TB

Improving a Tried and True Vaccine—BCG for the 21st Century

A jab with bacillus Calmette-Guérin (BCG) vaccine is one of the first experiences of life for most newborns around the world, says Marcus Horwitz, M.D., a tuberculosis vaccine researcher at the University of California, Los Angeles. Although not given routinely in the United States, BCG is the mostly widely administered vaccine in the world—some four billion people have been inoculated with the safe and easy-to-produce vaccine. Developed in the early 1900s by Frenchmen Albert Calmette and Camille Guérin, BCG is made from live, weakened *Mycobacterium bovis*, a close relative of the bacterium that causes TB in humans.

BCG does a good job of protecting infants from a form of TB that attacks the brain, but the vaccine's effects wear off over time and it probably prevents the common pulmonary form of TB only about half the time, notes Dr. Horwitz. Research in his lab centers on using genetic engineering to make a better BCG—what Dr. Horwitz calls "BCG-plus." Dr. Horwitz and his colleagues designed a vaccine that uses recombinant DNA technology to add a specific protein from *Mycobacterium tuberculosis* (*M. tb*), the bacterium that causes TB, to the basic BCG vaccine. The result, rBCG30, does a better job at protecting guinea pigs from TB than BCG, Dr. Horwitz found.

Guinea pigs are a good stand-in for humans in TB vaccine experiments for several reasons, says Dr. Horwitz. Unlike mice, another frequently used experimental animal, guinea pigs are naturally susceptible to infection by *M. tb* and readily develop disease in their lungs that resembles the disease in humans. Whereas mice used experimentally are typically highly inbred, guinea pigs used experimentally are generally outbred and thus possess a genetic diversity among individuals that mirrors that of humans. Dr. Horwitz published his findings about the effectiveness of rBCG30 in guinea pigs in 2000. The recombinant vaccine entered early stage human clinical trials in 2004.



An electron micrograph of *M. tuberculosis* residing within a human macrophage. The arrowheads indicate a major secretory protein of *M. tuberculosis*, which is expressed in large quantities in the vaccine rBCG30.
Credit: Dr. Horwitz

While a vaccine like rBCG30 might one day replace BCG as a vaccine given to infants, Dr. Horwitz and his colleagues are also working on a vaccine "booster" that could be used by the billions of people who have already received the standard BCG. In this strategy, the body's ability to detect and fight off invasion by *M. tb* is primed by BCG at birth, and then boosted by a later inoculation. Using the same *M. tb* protein as in the rBCG30 vaccine, the booster vaccine being designed by Dr. Horwitz is aimed at a subset of immune system cells called memory lymphocytes. Theoretically, a person who received a BCG prime and a boost with *M. tb* protein would be able to fight off infection by the TB bacterium by quickly producing large numbers of lymphocytes able to attack cells harboring the TB bacterium.

A third vaccine under development in Dr. Horwitz's lab is genetically engineered to be even safer than the time-tested BCG. The live, weakened strain of *M. bovis* in standard BCG is readily controlled by a healthy immune system, but can be dangerous for someone with a weakened immune system such as people infected with HIV. Dr. Horwitz has developed versions of BCG and rBCG30 in which the bacterium's ability to divide has been severely curtailed, which should make the vaccine safe even for people with a compromised immune system.

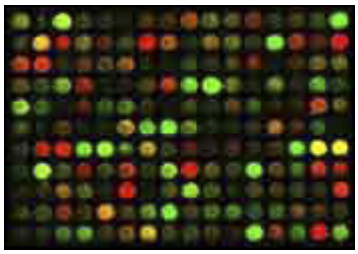
How Microarrays May Lead to Better Vaccines

Peter Small, M.D.

Peter Small, M.D., of Stanford University, is a molecular epidemiologist—a still-young field that combines genome studies with traditional epidemiological techniques in efforts to control diseases such as TB.

With colleagues at Stanford University's Center for Tuberculosis Research and other institutions, Dr. Small employs DNA microarray technology to detect genetic variations among strains of *Mycobacterium tuberculosis (M. tb),* the bacterium responsible for TB. What they are learning may lead to better treatments and vaccines for TB.

In the Chips



Pattern of gene activity on a microarray chip
Credit: Patrick Brown, Stanford University

DNA microarrays (also called gene chips) caused a sensation in science when they were introduced in the mid-1990s. The chips—tiny squares of glass containing hundreds of thousands of "spots" of DNA arrayed in a checkerboard pattern—give researchers a bird's-eye view of gene activity. Every spot in the grid is a short strand of DNA representing part of a known or suspected gene. Chopped up DNA pieces ("probes") taken from a cell are first attached to molecules that will make them fluoresce under a laser, then poured onto the chip. If a probe matches the DNA sequence of a spot, the two will stick to each other. Under a laser, the intensity of the glow reflects the numbers of copies of the DNA sequence. By analyzing the pattern of glowing spots, researchers see which genes are actively producing instructions for proteins. This, in turn, tells them something about the gene's function.

Along with collaborators including Thomas Gingeras, Ph.D., of the biotech company Affymetrix, Dr. Small used gene chips to see how various strains of *M. tb* differ at the genetic level.

TB does not affect every person equally. In most cases, an infected person's immune system successfully contains the organism within the lung. Sometimes, however, *M. tb* escapes containment and creates rampant, often deadly, TB. The Stanford team hypothesized that genetic characteristics of different strains of *M. tb* could explain some of the variation. Ultimately, Dr. Small hopes, it may be possible to "fingerprint" the particular strain of bacteria that a person has and tailor treatments precisely to that strain's weaknesses.

A first step towards that goal came when Dr. Small and colleagues performed microarray analysis on isolates of *M. tb* originally collected as part of a TB tracking study done in

San Francisco in the early 1990s. The investigators found deletions in the DNA of a dozen strains. In general, strains with more deleted gene segments were less likely to cause lung cavities. The researchers believe accumulated deletions may make *M. tb* less deadly.

Dr. Small's lab tested 200 clinical strains to see if the ones that cause the most severe disease have deletion patterns in common.

How the Shot Lost its Punch

In another study, workers in Dr. Small's laboratory used microarrays to make discoveries about the TB vaccine and gained new insights into why it may no longer be effective. The vaccine, BCG, was developed in the first decades of the 20th century. It contains live *Mycobacterium bovis*, the bacterium that causes TB in cattle. In the original vaccine, *M. bovis* was deliberately weakened enough so that the resulting vaccine would not cause disease, but would still provoke the immune system to mount a defense against future infection with *M. tb.*

Because good methods of storing bacteria were not available until well into the 20th century, public health workers had to grow their own supplies of BCG vaccine. Over time, the genetic contents of the vaccine and wild-type M. bovis diverged. Eventually, the vaccine changed so much that it could no longer effectively protect people against pulmonary TB.

However, the live bacteria in today's BCG vaccine still do prime the immune system. If a vaccinated person is ever tested for possible exposure to *M. tb,* the results will be positive. A clinician cannot tell if a positive result indicates exposure to *M. tb* itself or if the test is simply finding previous exposure to the related, but non-disease causing, BCG. For this reason, BCG is no longer administered in the United States, although it remains one of the most frequently given vaccines in other parts of the world.

DNA microarrays let the researchers see exactly how today's version of BCG differs from *M. bovis*—and from *M. tb.* The investigators showed how continued lost of genetic material in the vaccine over time could explain its reduced efficacy. They also found certain parts of the genome that are always present in *M. tb*, but that did not appear in BCG. Some of these key genome parts might account for *M. tb's* disease-causing ability. In the future, vaccines might be rationally designed to stimulate immune responses to those key bacterial genes.

Arraying machine
Credit: Patrick Brown, Stanford University

Closing In: The Long Road to a TB Vaccine

Steven Reed, Ph.D.

To cure TB worldwide would be a marvelous feat, to prevent it altogether would be even better. Currently only one TB vaccine exists. First administered in 1921, the BCG vaccine is safe, inexpensive and the most widely used vaccine in the world, according to the World Health Organization. There's only one thing wrong with BCG—it doesn't work very well. For children, BCG probably does prevent TB that infects the lining of the brain, but its ability to prevent adult pulmonary TB, the most common form of the disease, is doubtful.

Steven Reed, Ph.D., of the Infectious Disease Research Institute in Seattle, Washington, is trying to make a better TB vaccine. Vaccines work by showing the immune system a "preview" of certain parts (called antigens) of a microbe. The immune system learns what to expect, and if the actual disease-causing organism ever invades, the systems is primed to respond quickly.

Stimulating Memory

Mycobacterium tuberculosis (M. tb), the microbe that causes TB, lives inside immune system cells called macrophages and thwarts their infection-fighting ability. Because *M. tb* is so good at hiding inside the macrophages, some scientists think it may be impossible to prevent TB infection completely. A good vaccine, however, could keep infected cells to a minimum and thus prevent TB disease.

Child being vaccinated by Pan American Health Organization worker
Credit: Pan American Health Organization

Between 1995 and 2002, Dr. Reed and collaborators at the Seattle biotech company Corixa, in collaboration with the pharmaceutical company GlaxoSmithKline (GSK), identified specific *M. tb* proteins that trigger a strong reaction in immune system "memory" cells. When properly activated, memory cells confer long-lasting immunity. Using genetic engineering and recombinant DNA technology, Dr. Reed's group first created a single, artificial gene encoding the instructions for two proteins, and then produced sizable quantities of this so-called fused protein.

Adding Adjuvant

To create the immune-stimulating effect of a vaccine, proteins must be combined with an adjuvant. The only FDA-approved adjuvant now used in human vaccines, alum, boosts antibody production—one of two arms of the immune system. However, alum does not, however, boost the second, non-antibody, arm of the immune system, called cellular immunity. Cellular immunity, which includes memory T

cells, is what TB vaccine developers care about most. Creating adjuvants that can precisely drive the immune response an area of intense research.

With their fused protein and a new kind of adjuvant developed by **GSK**, and previously shown to be safe and effective in clinical trials, Dr. Reed and his colleagues built various vaccine constructs in an effort to find one that best stimulates the immune system. One, named Mtb 72f, entered the first stage of safety testing in healthy human volunteers in 2004.
Closing in

Closing In

"As recently as three years ago, no one thought we could improve on BGC enough to make a vaccine effort worthwhile," says Dr. Reed. "Now, we are closing in, and I really believe a new kind of vaccine—perhaps given as a booster to BCG—will be capable of preventing TB."

A Whiff of Protection: Mucosal Vaccines

Daniel Hoft, M.D., Ph.D. Credit: Dr. Hoft

To most people, vaccines mean needles. Daniel Hoft, M.D., Ph.D., of Saint Louis University in Missouri, is developing a different kind of vaccine—one that anyone fearful of a needle's jab should welcome. Dr. Hoft is working on a mucosal vaccine for TB that might be delivered as a squirt up the nose.

Most vaccines elicit a body-wide protective immune response. In contrast, mucosal vaccines target immune cells in the body's mucous membranes, such as the tissues lining the nose, mouth, lung, gut, and urogenital tract. Unlike watertight skin, mucosal surfaces allow substances to pass through. Oxygen and carbon dioxide move across the mucosa of the lungs as we breathe, for example. *Mycobacterium tuberculosis (M. tb)*, which lodge in the lower lung, take advantage of the openness of mucosa to invade our bodies, notes Dr. Hoft.

The current TB vaccine, BCG, limits the growth of TB bacteria in their actively dividing stage, but does not prevent infection itself. Also, BCG's protection appears to fade over time. This means vaccinated individuals who become infected may develop active disease when age or disease weaken immune responses and allow latent *M. tb* to reactivate, says Dr. Hoft.

The goal of a mucosal TB vaccine, he explains, is to stimulate sub-groups of immune system cells in the lung mucosa, thereby preventing or reducing infection in the first place. Because TB is so prevalent worldwide, Dr. Hoft adds, even a 50 percent reduction in infection rate would translate to enormous health benefits in endemic areas.

Dr. Hoft and his colleagues recently began research in mice on a vaccination strategy that combines a "prime" of one or more *M. tb* proteins delivered as a nose spray (the mucosal vaccine) with a "boost" of BCG. After determining which prime-boost combinations stimulate the most potent protective responses in the widest range of immune cells, the scientists will expose vaccinated mice to *M. tb* to test its effectiveness.

Beating the World's Most Successful Bug


William Jacobs, Ph.D.

Tuberculosis researcher William R. Jacobs, Jr., Ph.D., calls his foe, *Mycobacterium tuberculosis* (*M. tb*), the planet's most successful pathogen. The bacterium infects one-third of the world's population and can survive for decades inside immune system cells, called macrophages, that kill many other disease-causing organisms. But Dr. Jacobs, of Albert Einstein College of Medicine in New York City, says a detailed understanding of *M. tb* 's genetics is emerging from work in his and other labs and may lead researchers to new and more effective ways to prevent TB.

Dr. Jacobs has devised ways to find which genes *M. tb* must have to effectively invade and persist inside macrophages as well as the genes needed for robust growth inside the human host. Dr. Jacobs selectively mutates specific *M. tb* genes to create strains that cannot grow or persist well inside mouse models of TB. He and his colleagues are testing these mutants for their suitability as the basis of new kinds of TB vaccines.

With colleagues from the research labs at U.S. Food and Drug Administration in Rockville, MD, Dr. Jacobs recently published findings that a vaccine they made with mutant *M. tb* conferred long-term protection against TB in mice, including mice lacking CD4 cells, a key component of the immune system. The severely attenuated mutant *M. tb* strain lacked two specific genes that wild-type *M. tb* must have to successfully infect animal cells. The mutant strain is very safe to use in the mouse model infection and yet can confer long-term protection against TB infection.

The apparent effectiveness of this kind of severely attenuated live vaccine in immune-deficient mice is significant, say the scientists. Live, attenuated vaccines against such diseases as polio and measles have certain advantages over vaccines made with killed disease-causing organisms—they can generate immune responses that confer long-term protection against the disease after a single inoculation, for example. But vaccines containing live, weakened pathogens may not be safe for people whose immune systems are damaged, including those with HIV. The only existing vaccine against TB, BCG, is a live, attenuated vaccine that is not recommended for people with HIV.

Vaccines being developed by Dr. Jacobs and his colleagues are more severely weakened than the strain of TB in BCG and are weakened through the deliberate and specific

deletions of critical *M. tb* genes. The hope is that this will make these new vaccines safe enough for people with HIV or other conditions that impair immune health to use.

Global Problem, Global Plan

A Deadly Synergy

Christopher Whalen, M.D. Credit: Dr. Whalen

Tuberculosis weighs heavily upon the world and the poorest among us bear most of the burden. More than 80 percent of TB cases worldwide arise in only 22 countries, most of which are poor. Where malnutrition, substandard housing, and minimal health care are common, so too is TB. And where TB significantly impairs the health of many workers, entire economies are weakened.

The global crisis demands a global response. For decades, NIAID has supported research aimed at improving global health by lessening the burden of infectious disease. Key elements of NIAID's global health research plan include

- Disease prevention and treatment strategies adapted to specific needs of developing countries
- Sustained development of individual countries' research capacities
- Stimulation of scientific collaboration among individual researchers and initiation of partnerships among governmental and nongovernmental organizations.

When someone is infected with both *Mycobacterium tuberculosis (M. tb)*, the organism that causes TB, and HIV, the virus that causes AIDS, the combination produces a deadly synergy that makes both diseases more destructive together than either is alone. Nowhere is this terrible synergy more apparent than in Africa where, according to the World Health Organization (WHO), TB cases are increasing 10 percent each year in the wake increasing levels of HIV infection. In October, 2004, the WHO estimated that some 8 million Africans were co-infected by HIV and *M. tb.*

"We must achieve a better understanding of the HIV/AIDS-TB interaction so that African nations can make headway in controlling both these diseases," says Christopher Whalen, M.D., of Case Western Reserve University in Ohio.

While the exact mechanisms involved in the twin disease processes in people infected by both *M. tb* and HIV are unknown, it is possible that immune system chemicals released to fight reactivated TB also spur HIV replication, thus speeding the debilitating effects of AIDS.

In collaboration with Ugandan scientists in Kampala, Dr. Whalen is conducting clinical trials in HIV-positive people who are co-infected with TB. The overall aim of the research is to learn what mechanisms drive TB and HIV interactions.

One clinical trial tested the theory that a normal, robust immune response to TB infection has the undesirable effect of revving up the replication of HIV. The scientists gave trial participants a form of the common anti-inflammatory drug prednisone to blunt immune system activity against TB. They hoped the inexpensive drug would prevent TB reactivations. Although they could inhibit immune system activity that otherwise would spur on TB, the scientists also saw a rise in the amount of HIV circulating in the participants' blood. This finding, and the fact that prednisone is too toxic to give on a long-term basis, leads Dr. Whalen to believe that steroids like prednisone will not typically be part of the TB and HIV-fighting arsenal for most co-infected individuals. Nevertheless, he adds, immune system modulators of some kind probably will pay dividends in improved health for those infected with both *M. tb* and HIV.

In 2005, Dr. Whalen and his co-investigators in Uganda began enrolling patients in a new clinical trial. The scientists will test whether it is possible to delay the onset of full-blown AIDS in HIV-TB co-infected individuals by providing volunteers with antiretroviral drugs (that work against HIV) simultaneously with the normal 6-month-long regimen of TB drugs. As Dr. Whalen notes, if a course of antiretroviral drugs given before full-blown AIDS develops keeps HIV levels low enough that co-infected people can perform normal activities, the burden on the community's health care systems would be lightened. In addition to answering basic scientific questions about the interactions between HIV and *M. tb*, the Uganda trial could also help clinicians more effectively treat HIV-TB co-infected people.

"It's important to continue to study HIV/AIDS and TB," notes Dr. Whalen, "because the better we are at containing HIV, the better will become at containing TB. There will certainly be positive effects on both epidemics."

U.S.-South Korean Collaboration Will Advance TB Care

A project launched in 2003 that joins clinician-researchers at South Korea's National Masan Tuberculosis Hospital and an NIAID research team led by Clifton E. Barry, III, Ph.D., is set to improve tuberculosis (TB) diagnosis and treatment for the thousands of South Koreans who visit the hospital each year.

TB is a significant public health problem in South Korea, notes Dr. Barry. The national TB hospital in Masan treats some 5,000 patients annually. Half of the 1,000 inpatients each year suffer from multidrug-resistant TB (MDR-TB).

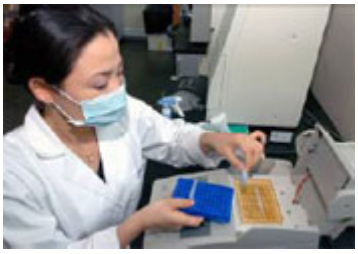
One of the first tasks of the NIAID-South Korean team, says Dr. Barry, was to improve the hospital's existing TB research labs and construct a new biosafety-level 3 (BSL-3) facility where research on MDR-TB can be conducted safely and efficiently. Set to open in March 2006, the BSL-3 lab will support Phase II clinical trials at the hospital testing new drugs against latent TB and against MDR-TB. All funding for the BSL-3 lab's construction was provided by the South Korean team.

Improved lab space will speed TB research in South Korea.
Credit: Tae Gwon Oh

Over the first three years of the joint project, the U.S. and South Korean researchers (led in South Korea by Seung-kyu Park, M.D., Ph.D., and Sang Nae Cho, DVM, Ph.D.) have conducted a variety of clinical research projects. For example, they have collected information about rates of MDR-TB in two groups of patients, those who are having an initial episode of active TB and those who have recurrent TB. The scientists are also studying bacterial and human genomes to learn if having certain gene variants makes an individual more likely to develop MDR-TB.

Two other clinical trials will be launched in 2006. In one, patients who have severe TB and who are scheduled to have a diseased portion of lung surgically removed will first receive a dose of a chemical that is absorbed by lung tissue. By studying this chemically-tagged lung tissue, the researchers hope to prove that TB bacteria persist in areas of lung where oxygen levels are low. The ultimate goal of this research is to identify drugs that work well against slowly growing TB bacteria in such low-oxygen environments.

The second clinical trial will enroll about 200 patients, half of whom will receive an experimental drug called metronidazole. Although this drug does not target actively growing and dividing TB bacteria, scientists believe that it does have some effect on non-dividing bacteria under low-oxygen conditions. Currently, there are no drugs to effectively treat and eliminate such non-dividing, persistent TB bacteria. By using high-resolution imaging technology to examine the patients during the trial, the researchers hope to learn quickly if the experimental drug is working, thus shortening the time it takes to evaluate the anti-tuberculosis activity of this and other experimental drugs. The metronidazole trial is based in part on research conducted by NIAID researcher Laura Via, Ph.D., who is developing a rabbit model of TB that can be used to test possible treatments for latent TB.



A researcher at South Korea's National Masan TB Hospital.
Credit: Seok Yong Lee

"We need a revolution in how we evaluate new anti-tuberculosis drugs," says Dr. Barry. "The goal is to develop effective treatment regimens for both drug-susceptible and drug-resistant forms of the disease and to cut down significantly on the time it takes to achieve a cure. To do that, we must be able to get good information quickly about how well the experimental compounds are working. With our colleagues in South Korea, we are poised to use the best available tools and thinking in battling MDR-TB there. It is a significant opportunity to address a major health crisis and to improve the lives of those who suffer from TB in South Korea and elsewhere."