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TB WARS

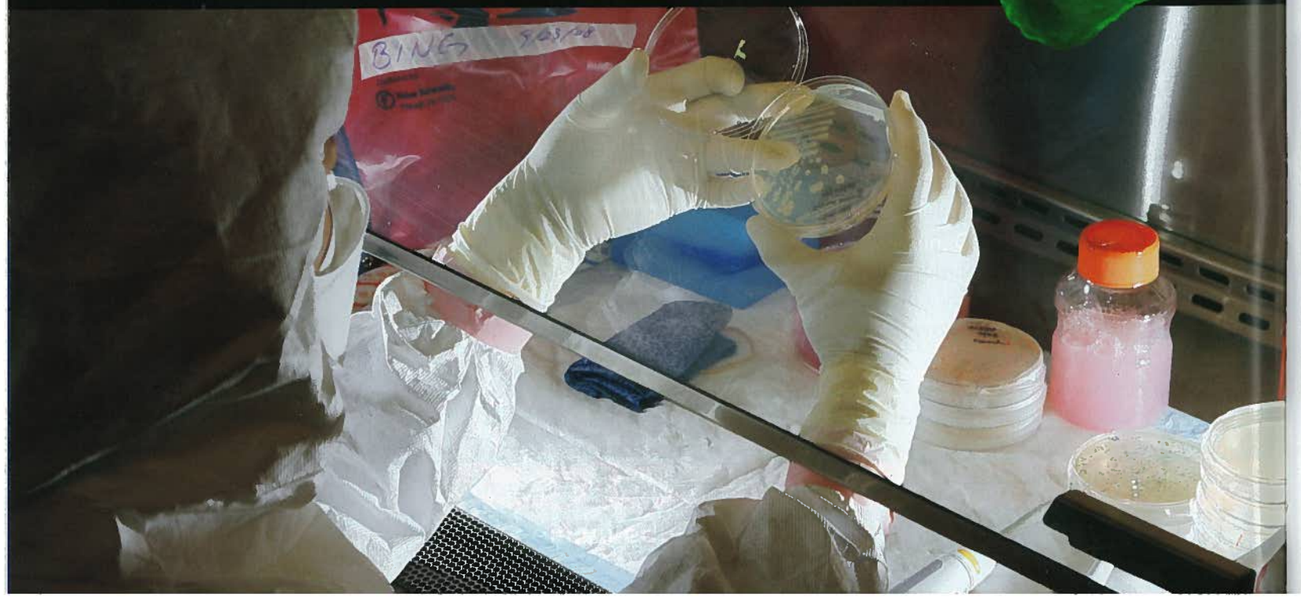
Major victories are likely as Einstein researchers attack tuberculosis on all fronts

Historic Bequest & Campaign page 34

TB WARS

BY GARY GOLDENBERG

Major victories are likely as Einstein researchers attack tuberculosis on all fronts



Mycobacterium tuberculosis, the bacterium that causes tuberculosis (TB), may well be the world's most successful pathogen. Today, it infects one-third of all people on Earth. The vast majority of them are only latently infected and suffer no ill effects, but that's hardly cause for comfort.

About one in 20 latent infections eventually progresses to active disease, with symptoms that include coughing, fever, fatigue and weight loss. If untreated, active TB kills about half its victims. Death can usually be prevented if active TB is caught early and properly treated—big "ifs" in the developing world, where most cases occur.

The numbers tell the story of TB's terrible toll: In 2011, TB sickened some 8.7 million people and took some 1.4 million lives, according to the World Health Organization. Africa, Russia, China and Southeast Asia have been especially hard hit.

At the risk of anthropomorphizing a microbe, *M. tuberculosis* is highly intelligent. It has figured out how to fend off all threats to its existence, from nature's immune cells to humankind's vaccines and medications. And the AIDS epidemic of the last 30 years has magnified TB's impact. In people co-infected with HIV and TB, immune systems weakened by HIV can no longer suppress TB. Co-infection has not only made TB more deadly but has greatly increased the number of active TB cases. Studies show that the risk of developing active TB is between 20 and 37 times greater among HIV-positive people; in some sub-Saharan countries, up to 80 percent of TB patients are infected with HIV.

Although scientists have come up with a variety of powerful mycobacterial-fighting antibiotics, the wily TB bacterium inevitably finds a way to evolve and resist the chemical onslaught. Some 650,000 people worldwide now have multidrug-resistant TB.

An even greater impediment to ridding the world of TB is the absence of an effective vaccine. Remarkably, after more than a century of research, there is still just one vaccine against TB: the Bacille Calmette-Guérin (BCG) vaccine, prepared from a strain of the bovine tuberculosis bacillus, *Mycobacterium bovis*. The live attenuated (weakened) BCG vaccine has been used in humans since 1921 and has proven notoriously inconsistent in protecting against TB.

At Einstein, home to one of the country's most comprehensive TB research programs, developing better TB vaccines is a top priority. Two teams of Einstein scientists are working on novel TB vaccines that may one day replace the BCG vaccine. Einstein researchers are also deciphering *M. tuberculosis*' secrets gene by gene, seeking vulnerabilities in the TB genome that could be targeted by new drugs. And for countries with raging TB epidemics, Einstein scientists are devising better

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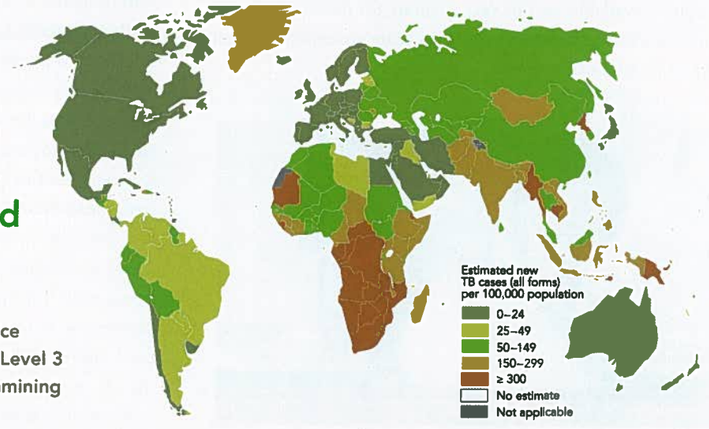


Bing Chen, M.D., director of Einstein's Price Center/Block Research Pavilion Biosafety Level 3 Laboratory, sits at a biosafety cabinet examining colonies of *Mycobacterium tuberculosis*.

A long-sought global health goal is a simple, quick and inexpensive test for active TB infections that threaten patients' health

An assay that detects three antibodies has shown promise for quickly and inexpensively diagnosing active TB infections. The assay was based on the discovery that TB bacteria actively infecting human lungs shed tiny pieces of their membrane vesicles into the bloodstream, causing patients to produce three different antibodies in response.

strategies for caring for patients, and developing tests that rapidly detect active TB infections and indicate which antibiotics will work best for each patient.

In this battle between man and microbe, *M. tuberculosis* still has the upper hand—but maybe not forever. Thanks to the efforts of Einstein researchers, TB may one day join smallpox on the short list of extinct microbial killers.

Quick, Simple and Inexpensive: The Holy Grail in TB Diagnosis

Most people infected with TB worldwide have latent infections, meaning the TB bacteria in their bodies are controlled by their immune systems and are not causing disease. A long-sought global health goal is a simple, quick and inexpensive test for *active* TB infections, in which multiplying bacteria overwhelm the immune system, threatening patients' health and posing a risk for spreading the infection.

Such a test is especially needed in poor countries, where the vast majority of cases occur. But the simple and affordable tests now available are not very accurate for detecting active infection, and tests that are more accurate are expensive, complex or time-consuming.

Jacqueline M. Achkar, M.D., M.S.

Sputum-smear microscopy is now the most commonly used point-of-care test for active TB infection. A patient coughs up a sputum sample, which is smeared onto a glass slide and stained with a dye. If *M. tuberculosis,* the microbe that causes TB, is visible under the microscope, the patient almost certainly has an active infection.

The problem with this test is that someone with no observable bacteria in the sputum can still have an active TB infection. "Only about half of all people with active infections have detectable *M. tuberculosis* in their sputum smears," says **Jacqueline M. Achkar, M.D., M.S.**, associate professor of medicine (infectious diseases) at Einstein and an attending physician in medicine at Montefiore, the University Hospital and academic medical center for Einstein. "So people with active infections but who are smear-negative often aren't diagnosed."

This isn't such a problem in developed countries. "In the United States, if the clinical signs and symptoms point to TB, we usually don't stop at negative sputum smears," Dr. Achkar explains. "But in resource-limited settings, people with negative smears would get sent home until they get so sick that they become smear-positive. Obviously, that's not ideal."

A possible alternative test would look not for *M. tuberculosis* itself but for antibodies the immune system makes in response to an active infection. This approach has been tried, but with disappointing results. "Many scientists believe that *M. tuberculosis* doesn't produce a significant antibody response, but new evidence shows otherwise," says Dr. Achkar. "It's likely that people have been looking for the wrong antibodies." She may now have found the right ones.

Dr. Achkar's Einstein colleagues previously discovered that when *M. tuberculosis* multiplies in the lungs, the bacteria shed tiny pieces of their membranes into the bloodstream, making the infection more virulent and triggering a small but detectable antibody response—at least in mice.

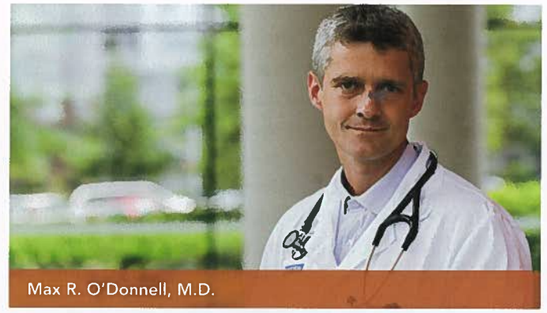
Dr. Achkar then showed that these bacterial "membrane vesicles" triggered the same effects—but with even more pronounced antibody production—in people with active TB. She found that these patients produce antibodies against three specific types of vesicle proteins, suggesting that this antibody trio might serve as a highly sensitive and specific biomarker for active TB infection.

Dr. Achkar recently devised an assay for the three antibodies and tested its accuracy in 28 patients known to have active TB infections, plus a control group of 16 people with latent TB. Among the actively infected patients, the test correctly diagnosed all 16 smear-positives. And—particularly important—the antibody test was positive for 9 of 12 smear-negatives (people actively infected but with no *M. tuberculosis* in their sputum). No one in the control group tested positive.

"If we can sustain these numbers in large-scale testing and turn our assay into a simple 'dipstick' blood test, that would be phenomenal," she says.

A New Gold Standard for Diagnosing TB?

The TB news from South Africa has long been downbeat. Yet **Max R. O'Donnell, M.D.**, assistant professor of medicine (pulmonary medicine) and of epidemiology & population health at Einstein, remains optimistic.



Max R. O'Donnell, M.D.

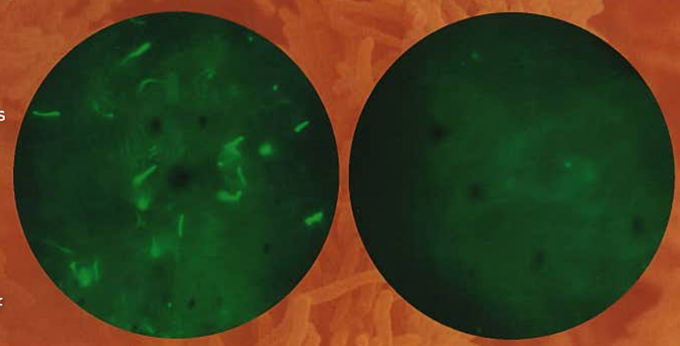
"It must be my personality," says Dr. O'Donnell, who studies the nexus of the TB and HIV epidemics that are both surging in South Africa—particularly in KwaZulu-Natal province, where he works. "But I've seen great improvements in combating HIV in South Africa. When I started working there more than a decade ago, perhaps 13 percent of HIV-infected patients were getting anti-retrovirals. Today, it's up to 80 percent, and people are living much longer. I'm hopeful we'll eventually achieve the same results with TB through some combination of prevention and treatment."

Today, Dr. O'Donnell, also an attending physician in medicine at Montefiore Medical Center, participates in efforts to improve TB care in South Africa, including the first clinical trial of the fluorophage diagnostic test. This test, which could dramatically improve the diagnosis of TB and drug-resistant TB, was developed in the lab of Einstein's William R. Jacobs, Jr., Ph.D., professor of microbiology & immunology and of genetics. Dr. O'Donnell organized the clinical trial that is evaluating the diagnostic test in South Africa.

THE FLUOROPHAGE TEST FOR DETECTING TB AND DETERMINING DRUG SENSITIVITY

Left, rapidly detecting TB: A sputum sample was incubated with viruses (phages) that specifically infect TB bacilli and deliver the gene for green fluorescent protein. A few hours later, each living TB cell infected with a phage expresses the green fluorescent gene and glows, indicating an active TB infection.

Right, assessing drug sensitivity: The anti-TB drug rifampicin was added to the sputum sample at left. Twelve hours later no glowing bacteria are visible, indicating that this strain of TB is sensitive to the drug.



Images by Paras Jain, Ph.D., and Neil Eisenberg, M.D.

The trial, launched last November, will involve some 350 patients with suspected TB at the Prince Cyril Zulu Communicable Diseases Clinic in Durban, the largest city in KwaZulu-Natal province. The fluorophage test will be compared with two other TB tests: sputum-smear microscopy and the GeneXpert MTB/RIF test (which analyzes the microbes' DNA to gauge antibiotic sensitivity). The former is the standard test for diagnosing TB, while the latter is the most reliable way of detecting drug-resistant TB.

Dr. O'Donnell expects that the fluorophage test will be more sensitive than the sputum-smear test (which misses half of all patients with active TB), and more clinically useful than the GeneXpert test (which is good at detecting drug-resistant TB strains but cannot distinguish between active and latent bacteria).

Better TB diagnostics would be a godsend in areas such as KwaZulu-Natal. Of the province's 10 million residents, some 100,000 have TB at any given time. Perhaps 5 percent of those, or 5,000, have drug-resistant TB. (To put those numbers into perspective, New York City, with just over eight million residents, has only 600 yearly cases of TB, with a mere handful that are drug resistant.)

Adding to KwaZulu-Natal's woes, about 70 percent of people with TB are co-infected with HIV. "If TB is fire, HIV is gasoline," says Dr. O'Donnell. "We need new tools to keep both epidemics in check, and a better diagnostic test for TB would certainly help."

The fluorophage test may also be useful for assessing how TB infections respond to treatment. "Now, therapy for drug-resistant TB is given for 18 to 24 months, and then we wait another two years before we declare a patient disease free," says Dr. O'Donnell. "With the fluorophage test, we could have an ongoing measure of the patient's TB infection and adjust the patient's therapy accordingly."

Go South, Young Man

Everyone should take an occasional break. So in 2004—after 12 years of study and training—**James C. M. Brust, M.D.**, assistant professor of medicine (general internal medicine and infectious diseases) at Einstein, swerved from the usual career track before starting a fellowship in infectious diseases. He traveled south, not to some Caribbean resort but to KwaZulu-Natal, ground zero for both HIV and tuberculosis.

Dr. Brust worked on optimizing treatment for people with HIV, many of whom were co-infected with TB. Anti-HIV drugs were working well against HIV in co-infected patients, but the drugs for TB were not. Hundreds of these patients failed to respond to first-line TB medications, eventually falling gravely ill or dying.

"The numbers were bewildering," he says. Multidrug-resistant TB (MDR-TB) and its less common cousin, extensively drug-resistant TB (XDR-TB), were just beginning to take hold in South Africa. It would be another two years until the full scope of the problem would come to light, which happened thanks to the work of Neel Gandhi, M.D., then an Einstein faculty member.

Dr. Brust resumed his training after that year abroad. But he would return time and again to KwaZulu-Natal to try to figure out how to combat MDR-TB. The care administered was too centralized. Rural patients with MDR-TB had to travel long distances for treatment and remain hospitalized for months—assuming beds were even available.

"After discharge, people would go back home and default on treatment, and hospitals didn't have the staff to chase them down," says Dr. Brust, who is also an attending physician in

In Dr. Brust's home-treatment program, pickup trucks deliver TB medication—and nurses to administer it—to the doorsteps of patients in need. Below, preparing an injection.

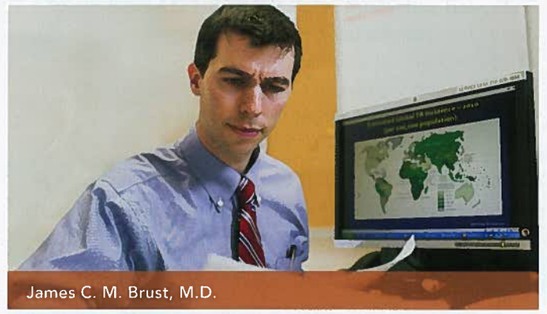
Hundreds of patients failed to respond to first-line TB medications, eventually falling gravely ill or dying

A DRUG-RESISTANT TB PRIMER

Multidrug-resistant TB (MDR-TB): TB that does not respond to isoniazid and rifampicin, the two most potent anti-TB drugs. Increasing numbers of TB cases are being diagnosed as MDR-TB. Compared with first-line treatment, second-line drugs for treating MDR-TB require a longer course and are more toxic, more costly and not readily available in resource-limited settings.

Extensively drug-resistant TB (XDR-TB): TB that is resistant to rifampicin and isoniazid, as well as to any member of the quinolone family of antibiotics and at least one of four second-line injectable anti-TB drugs.

- The World Health Organization calls MDR-TB "a major public health problem" and estimates that there are 650,000 cases worldwide. About 9 percent of these patients have XDR-TB. Only a small proportion of drug-resistant cases are detected and treated appropriately.
- Resistance to anti-TB drugs can occur when these antibiotics are misused or mismanaged—for example, when patients fail to complete their full course of treatment; when healthcare providers prescribe the wrong treatment, dose or length of time for taking the drugs; when the supply of drugs is not always available; or when the drugs are of poor quality.



James C. M. Brust, M.D.

internal medicine at Montefiore. It was the perfect scenario for cultivating drug-resistant strains of TB.

Dr. Brust's solution was to bring the care to the patients. After joining the Einstein faculty in 2008, he spearheaded an effort to develop a rural, home-based treatment program for people infected with MDR-TB (including those co-infected with HIV) in Tugela Ferry, a rural and very poor part of KwaZulu-Natal where 200,000 people lived. Under this pilot program, nurses visited patients at home and gave injections while community health workers and family supporters were trained to help patients stick with their treatment plans and monitor adverse reactions. Physicians provided additional care at monthly follow-up visits. Patients also received HIV care as needed.

The program has since been adopted at other KwaZulu-Natal hospitals and may soon be used widely in South Africa. In 2010, Dr. Brust received a five-year grant from the National Institute of Allergy and Infectious Diseases to formally evaluate the program. A preliminary analysis of 80 MDR-TB patients (66 of them co-infected with HIV) found that 95 percent stayed with the program and 77 percent were cured, as reported in the *International Journal of Tuberculosis and Lung Disease* in 2012. If successful, the program could become a new model for TB treatment in resource-limited settings.

Between trips to South Africa, Dr. Brust sees patients at Montefiore who have a wide variety of infectious diseases, including HIV, pneumonia and malaria. Fortunately, very few have TB. "Maybe one day," he says, "we can say the same about people in KwaZulu-Natal and beyond."

Attacking Drug-Resistant TB with a One-Two Punch

In 2006, an Einstein-led research team working in KwaZulu-Natal made an alarming discovery: rates of drug-resistant tuberculosis had increased fivefold since the beginning of the decade—far faster than anyone had expected. What was happening in KwaZulu-Natal was not an anomaly but a regional bellwether, adding to the already enormous challenge of keeping the southern African TB epidemic under control.

Patients infected with MDR-TB do have treatment options, thanks to so-called second-line TB drugs. However, few if any of those drugs work against MDR-TB's deadly relative—XDR-TB. In the KwaZulu-Natal study, for example, 52 out of 53 XDR-TB patients died from the disease.

But there may be some relief in sight, thanks to Einstein research. In early 2009, **John S. Blanchard, Ph.D.**, the Dan Danciger Professor of Biochemistry, reported in *Science* that a combination of two drugs (clavulanate and meropenem) was

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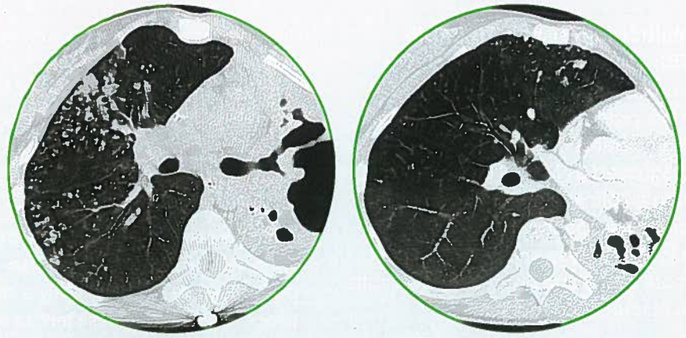


A May 2009 CT chest scan, left, on 14-year-old Chechnyan girl shows bilateral diffuse pulmonary TB that was extensively drug resistant. She was prescribed antibiotics, including the two-drug combination therapy (clavulanate plus meropenem) devised by Einstein's John Blanchard, Ph.D., and underwent surgical removal of her left lung. A scan four months later, right, shows significant clearing of affected (white) areas in the right lung. She is now a healthy young woman, her Belgian physician reports. (Images courtesy of Marie-Christine Payen, M.D.)

highly effective in inhibiting the growth of drug-susceptible as well as XDR strains of TB in laboratory culture.

Individually, the drugs have little or no effect on *M. tuberculosis*, but in combination they're potent. Clavulanate inhibits a bacterial enzyme (beta-lactamase) that shields TB bacteria from meropenem, a member of the beta-lactam class of antibiotics.

Dr. Blanchard didn't originate the idea of inhibiting beta-lactamase to make beta-lactam antibiotics effective. His contribution—the result of years spent studying mycobacterial enzymes—was finding the ideal combination of beta-lactamase inhibitor and beta-lactam antibiotic that could overpower *M. tuberculosis*. Both drugs have already been approved by the Food and Drug Administration for fighting other bacterial infections. And while they haven't yet been tested together in clinical trials, there is evidence for the combo's clinical effectiveness.

In 2010, physicians at L'Hôpital Universitaire Saint-Pierre in Brussels, Belgium, used the two-drug regimen in a last-ditch effort to save the life of a 14-year-old girl from Chechnya. The acutely ill and malnourished patient was diagnosed with XDR-TB after failing to respond to standard first- and second-line TB medications. "We had nothing to lose," says the lead physician, Marie-Christine Payen, M.D.

Four weeks after the combination therapy was begun, the girl's health started improving. And after 11 weeks, her sputum tests were negative for TB. The medical team reported its treatment's success in the *Pediatric Infectious Disease Journal*.

Last year, Dr. Payen used the two drugs, together with one or two second-line drugs, to treat six more XDR-TB patients. Five of the patients showed dramatic improvement, according to a paper in the *International Journal of Tuberculosis and Lung Disease*. The Belgian team has since used the regimen to treat another six patients, "with some success," Dr. Payen tells *Einstein* magazine.

"Dr. Payen has shown that six months of this therapy can cause TB bacteria in sputum and the circulating blood to fall from significant levels to zero," says Dr. Blanchard. "That would take 18 months on second-line therapy, if it worked at all."

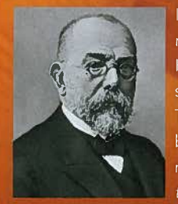
This spring, Dr. Payen began a 30-patient Phase II clinical study of the drug combination. Meanwhile, GlaxoSmithKline, a London-based pharmaceutical company, is leading a consortium of 12 international partners in a major, multiyear study of various beta-lactam therapies for TB, including the meropenem-clavulanate combination. The trial will take place in South Africa.

John S. Blanchard, Ph.D.

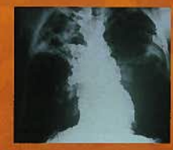
THE ABCs OF TB

TB primarily affects the lungs (pulmonary tuberculosis) and spreads mainly through inhalation of bacteria sneezed or spit into the air. Victims die because the bacteria progressively destroy lung tissue.

TB bacteria also target the bones and joints, the genitourinary system, the central nervous system, the intestines and the lymph nodes.



In 1882, the German microbiologist Robert Koch showed conclusively that pulmonary TB was caused by a bacterium, which he named *Mycobacterium tuberculosis* because of the tubercles it causes—hence the name tuberculosis.



William Roentgen's discovery of X-rays in 1895 allowed physicians to monitor the progress of patients' TB.

Solid Comfort, by S. R. Stoddard, courtesy of the Trudeau Institute Archives

The Hunchback Of Notre Dame, (1939)

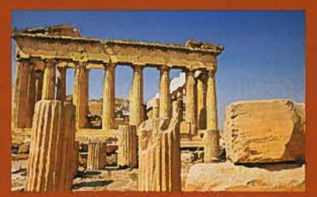
The spinal curvature caused by TB was known as Pott's disease (after the English physician Percival Pott, 1714–1788). Victor Hugo's famous Hunchback of Notre Dame exhibited the kind of deformity that can be caused by Pott's disease.

TB is an ancient disease. Tubercles detected in prehistoric bones indicate that TB has affected people since at least 5800 BCE. Evidence of TB infection has turned up in Egyptian mummies and in ancient burial sites in China and throughout Europe.

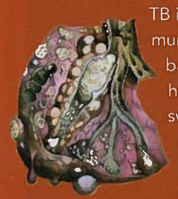
HIP / Art Resource, NY

Dating back to the fifth century, TB that infects and inflames lymph nodes in the neck (which can result in severe ulceration and scarring) was referred to as the King's Evil because the touch of kings or queens was thought to cure victims. Philip Augustus of France (1180–1223) touched 1,500 people during a single ceremony.

The 19th-century New England folk belief in vampires centered on fear that TB victims could return from the dead as vampires and cause surviving relatives to develop the disease.



The ancient Greeks referred to TB as phthisis (literally, "wasting")—a term used by physicians for more than 2,000 years. Hippocrates called phthisis the most widespread disease of his age, and phthisis was the cause of death listed on Charlotte Brontë's death certificate. Until it was called tuberculosis, the standard lay term for TB was consumption, because victims seemed to be consumed by the disease.



TB infection triggers immune cells to wall off the bacteria from surrounding healthy tissue, forming swollen regions known as granulomas. Lung granulomas were referred to as tubercules.

Koch proved that TB is caused by microbes but wasn't the first to suspect as much. The English physician Benjamin Martin, author of *A New Theory of Consumption* (1720), speculated that the disease resulted from the actions of "wonderfully minute living creatures."

TB sanatoriums debuted in Germany in the 1850s to provide TB patients with fresh air and good nutrition. They were the first major advance in treating the disease. Einstein's Van Etten Building was built in the 1950s as a 500-bed TB sanatorium but was never used for that purpose, thanks to a sharp decline in U.S. TB cases due to the introduction of effective drug therapy and other public health measures.

A critically ill TB patient was first treated with streptomycin on November 20, 1944—with dramatic results. The drug was considered the long-sought magic bullet for TB, but streptomycin-resistant TB strains soon developed.

In 1987, the American Medical Association's Advisory Council for the Elimination of TB predicted that the disease would be eliminated worldwide by 2010.

In 2013, the World Health Organization stated that someone dies from TB every 15 seconds.

P. J. Maglione and John Chan, M.D.

Arturo Casadevall, M.D., Ph.D., and Ph.D. candidate Lisa Brown.

Rafael Prados-Rosales, Ph.D.

B Cells, the Forgotten Weapon

In tuberculosis research, B cells get no respect.

"B cells got a bad rap more than a century ago when physicians began experimenting with serum therapies for TB," says **John Chan, M.D.**, professor of medicine and of microbiology & immunology at Einstein and attending physician in infectious diseases, department of medicine, at Montefiore.

The blood's serum contains antibodies, which are produced by immune cells called B cells. The idea behind serum therapy is to protect people against infection.

This approach worked well against some infectious diseases. But studies in the late 1800s found that antibodies offered inconsistent protection against TB, leading to skepticism regarding their usefulness.

Dr. Chan and his Einstein colleagues are among the small coterie of TB investigators who believe not only that B cells deserve a second look but that they may be essential for creating better TB therapies and vaccines that are more effective.

Dr. Chan first got interested in B cells after noticing unusual aggregates of cells in the lungs of mice infected with *M. tuberculosis*. To his surprise, those collections of cells were chock full of B cells.

"The body's immune response to TB was supposedly driven by T cells, not B cells," he says. "But we saw that humans with active TB had the same B-cell aggregates in lung tissue as mice did, which led us to wonder, 'What are those B cells doing?'"

Plenty, it turns out. In studies of mice, Dr. Chan and **P. J. Maglione,** an M.D./Ph.D. student in his lab, showed that B cells:

- form a significant part of lung granulomas (clumps of immune cells that create a physical barrier against the spread of bacteria);
- modulate the functions of T cells, which are critical in defending against *M. tuberculosis*;
- regulate the inflammatory response in the lungs of infected hosts; and
- boost the effectiveness of the BCG vaccine, the only approved TB vaccine.

"While more needs to be learned about the role of B cells in TB, there are already enough data to suggest that this arm of immunity should not be overlooked," says Dr. Chan. Today, he and his colleagues are in the middle of a five-year study, funded by the National Institute of Allergy and Infectious Diseases, to shed more light on how B cells help fight TB.

Immunofluorescence staining reveals a B-cell aggregate in a patient with pulmonary tuberculosis. The surfaces of the B cells are stained red and the nuclei are stained blue.

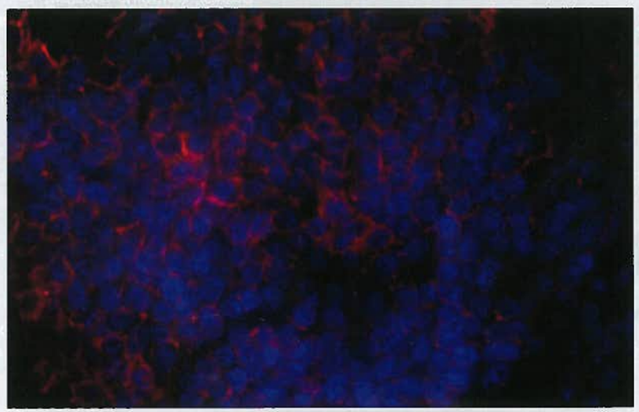
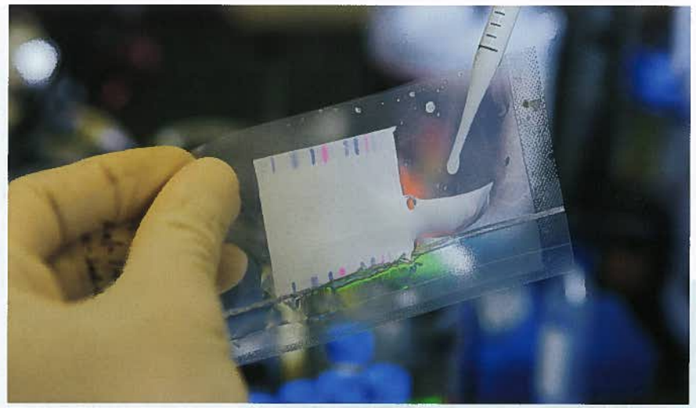


Image by Soumya Chakravarty, M.D., Ph.D.

B cells got a bad rap more then 100 years ago. But they may be essential for creating better TB therapies and vaccines that are more effective

The Casadevall team is trying to identify the parts of the vesicles that elicit immunity and use them as the foundation for a vaccine



Dr. Chan is not the only Einstein TB researcher interested in antibody-producing B cells. **Arturo Casadevall, M.D., Ph.D.**, professor and chair of microbiology & immunology, professor of medicine, the Leo and Julia Forchheimer Chair in Microbiology and Immunology and director of Einstein's Center for Immunological Sciences, is working on two different TB vaccines, a vesicle vaccine and a conjugate vaccine—both aimed at spurring B cells to kill *M. tuberculosis* before it gains a foothold in the lungs. (By contrast, virtually all the other TB vaccines under development try to provoke a response from the immune system's T cells.)

Vesicle TB vaccine. In 2011, **Rafael Prados-Rosales, Ph.D.**, a postdoctoral fellow in Dr. Casadevall's lab, observed that TB bacteria grown in laboratory culture release tiny pieces of their membranes. Studies in mice showed that these membrane vesicles transport proteins and lipids that promote the virulence of *M. tuberculosis* in the lungs. Vaccinating mice with these vesicles elicited a modest immune response, equal to that provoked by the BCG vaccine.

"Our next challenge is to strengthen that response," says Dr. Casadevall, also attending physician in medicine (infectious diseases) at Montefiore. "One reason we're not getting a better antibody response is that the vesicles are too heterogeneous. We're working with Bill Jacobs' lab to engineer TB strains to overproduce vesicles that are more homogenous." At the same time, the Casadevall team is trying to identify the exact parts of the vesicles that elicit protective immunity, and then use those antigens as the foundation for a vaccine.

A vesicle-based vaccine would have an important advantage over BCG, a live vaccine that can cause complications that include meningitis (inflammation of the brain's outer covering).

Conjugate TB vaccine. Dr. Casadevall's conjugate vaccine combines a polysaccharide (a long chain of carbohydrate molecules) from the outer cell wall of *M. tuberculosis* with an unrelated foreign protein.

"Polysaccharides are not very immunogenic, which means that they don't elicit much of an antibody response," he explains. "So you have to trick the immune system, and one way to do that is to attach a part of the bacterium's polysaccharide to a protein that is highly immunogenic. The immune system responds by creating a robust antibody response to the polysaccharide."

The researchers are currently experimenting with a vaccine consisting of a TB polysaccharide called arabinomannan and a nontoxic immunity-bolstering protein derived from anthrax bacteria.

Einstein is the only research center working on B-cell TB vaccines. "Sometimes it's very hard for the research community to switch gears," says Dr. Casadevall, whose vaccine studies are funded by grants from the Bill and Melinda Gates Foundation. "The data in support of B cells are there, and they have been confirmed by other labs. But it can take many years to change people's thinking."

Dr. Casadevall acknowledges that defeating TB may require both B-cell and T-cell vaccines. "I'm agnostic in this regard," he says. "What matters to me is to get a vaccine that works. I don't particularly care how it does it."

The Persister

Williams R. Jacobs, Jr., Ph.D., is obsessed with 0.1 percenters. Not the earners at the top of the income pyramid, but the tiny minority of tuberculosis bacteria that seemingly survive any and all attackers.

"If you treat *M. tuberculosis* with a powerful antibiotic like isoniazid, you kill 99.9 percent of the cells in the first four or five days," says Dr. Jacobs, professor of microbiology & immunology and of genetics at Einstein and a Howard Hughes Medical Institute investigator. "But you just can't kill that last 0.1 percent. Whether confronted by drugs or immune cells, these 'persisters' are able to activate a genetic program involving hundreds of genes that allows the bacteria to survive."

If TB bacteria are persistent, they've met their match in Dr. Jacobs. For the better part of three decades—all of them spent at Einstein—he has studied these stealthy microbes, finding new and creative ways to exploit their vulnerabilities.

Dr. Jacobs is a pioneering TB researcher who first made his mark on the field in 1987, when he figured out how to manipulate the TB microbe's genome. He did so using mycobacteriophages (or "phages," for short), viruses that specifically infect mycobacteria. The phages he engineered were able to penetrate *M. tuberculosis*' tough, waxy envelope and insert new mycobacterial DNA.

The word "revolutionary" is overused, but this discovery transformed TB research. Dr. Jacobs' phages helped reveal how isoniazid (a first-line TB medication) disables TB bacteria. Later, he determined the genetic reason that the BCG strain of TB (used in the first and only TB vaccine) triggers an immune response but does not cause full-blown infection.

More recently, Dr. Jacobs created a rapid fluorophage test that both diagnoses the presence of TB in a sputum sample and determines whether that particular strain is susceptible to antibiotics. To do so, he took phages that infect TB bacteria and engineered them to carry a fluorescent gene taken from fireflies.

Each mycobacterial cell infected with the virus expresses the fluorescent-protein gene, causing it to reveal its presence by glowing green under the microscope (signaling that the patient has an active infection). Upon exposure to antibiotics, the bacterial strain will remain glowing if it's drug resistant, but the green signal will fade away (along with the bacteria themselves) in the case of an antibiotic-sensitive strain. In 36 hours, the simple and inexpensive fluorophage test can pinpoint MDR-TB and XDR-TB strains. Dr. Jacobs described his fluorophage test in the *Journal of Clinical Microbiology* in 2012. (See page 23 for images of the test.)

Whether confronted by drugs or immune cells, persisters are able to activate a genetic program involving hundreds of genes that allows the bacteria to survive

In July, Einstein's William R. Jacobs, Jr., Ph.D.; Michelle Larsen, Ph.D.; Paras Jain, Ph.D.; and Oren Mayer taught the 6th Annual Mycobacterial Genetics Course at the KwaZulu-Natal Research Institute for Tuberculosis and HIV (K-RITH) in Durban, South Africa. "This is how we're educating the next generation of African scientists," says Dr. Jacobs.

If the test proves successful in clinical trials, it could save countless lives in places such as sub-Saharan Africa and Central Asia, where drug-resistant TB strains are a growing concern—and where tests to diagnose active TB infections and determine their drug sensitivity are too costly and technologically complex to be used.

Phages are also playing a role in Dr. Jacobs' promising new TB vaccine, dubbed IKEPLUS. It's based on an ingenious transfer of genes from *M. tuberculosis* to *M. smegmatis*, a closely related mycobacterial species that is lethal to mice at high doses but does not harm people. To construct the vaccine, Dr. Jacobs and his colleagues first created a version of *M. smegmatis* lacking a set of genes, known as ESX-3, that enable the bacteria to evade host immunity. (This strain was designated IKE, for "immune killing evasion.")

When high doses of the ESX-3-deficient *M. smegmatis* bacteria were infused into mice, the bacteria quickly succumbed to their hosts' immune systems via a robust T-cell response—the same response a successful TB vaccine would elicit.

Unfortunately, removing the same set of genes from *M. tuberculosis* killed the bacterium—which meant that *M. tuberculosis* could not be manipulated in this way to make a live vaccine. But the Jacobs team found a workaround. They took the *M. smegmatis* bacteria lacking ESX-3 and inserted into them the analogous set of *M. tuberculosis* ESX-3 genes. These viable *M. smegmatis* bacteria, called IKEPLUS, were then infused into mice, which fought off the infection as before. Eight weeks later, the mice were challenged with high doses of *M. tuberculosis*, which kills mice as well as people. These "vaccinated" mice lived two and a half times longer than control mice, as Dr. Jacobs reported in 2011 in *Nature Medicine*.

Equally impressive, says Dr. Jacobs, was the markedly reduced level of TB bacteria found in the animals' tissues. "Most notably," he said, "those vaccinated animals that survived for more than 200 days had livers that were completely clear of TB bacteria, and nobody has ever seen that before." Just one in five mice showed this robust response in the initial experiments, indicating that IKEPLUS must be improved before it can be considered for clinical trials.

In yet another phage-related experiment, Dr. Jacobs and his colleagues are systematically deleting all 4,500 genes in the TB bacterium, one gene at a time. In this way, the researchers hope to learn more about the function of each gene by observing how TB bacteria fare without it.

"We hope our systematic evaluation of the entire TB genome will reveal vulnerabilities that we can target with new and more effective treatments and vaccines," says Dr. Jacobs, whose work is funded in part by the National Institutes of Health and the Bill and Melinda Gates Foundation.

Quite fittingly, Dr. Jacobs' first phages were discovered in the Bronx—in a soil sample from his own backyard. He has also found other useful phages at the Bronx Zoo, many with the help of local high school students whom he sponsors in a summer science program called "No Phage Left Behind."

Dr. Jacobs' enthusiasm for basic research is, well, infectious. Quite a few of his summer students have gone on to careers in science. One recent "student" was Bill Gates, who invited Dr. Jacobs to his Manhattan office in 2012 so he could learn more about research into TB. Dr. Jacobs ended up spending two hours educating the software pioneer turned global health philanthropist. "He loved it," says the researcher.

Steven A. Porcelli, M.D.

The Accidental Tuberculologist

The advice that **Steven A. Porcelli, M.D.**, would offer aspiring researchers boils down to three words: follow your nose. "Sniff out what's interesting and go after it," he says. "You never know what you might find."

Dr. Porcelli's own career, which began in rheumatology, is a perfect illustration. In the 1990s, he was studying the autoimmune disease lupus when he observed some mysterious T cells that seemed to behave differently from other known T cells. "Having found them, I thought I should figure out what they do," says the physician-scientist, who is now professor of microbiology & immunology and of medicine (rheumatology) and the Murray and Evelyne Weinstock Chair in Microbiology and Immunology at Einstein.

Using *M. tuberculosis* as his model pathogen, Dr. Porcelli discovered that the T cells he was studying can recognize lipid antigens. Until then, scientists thought that T cells could recognize only protein antigens.

This insight would change immunology—and Dr. Porcelli's career. "Those T cells really got me thinking about tuberculosis," he says. "Since the *M. tuberculosis* bacterial cell is about one-third lipid by weight, it made sense that our immune systems would have evolved a way to recognize the lipids of foreign bacteria. So if we have these T cells that attack *M. tuberculosis* by recognizing its lipids, why can't we eliminate *M. tuberculosis* from the body?"

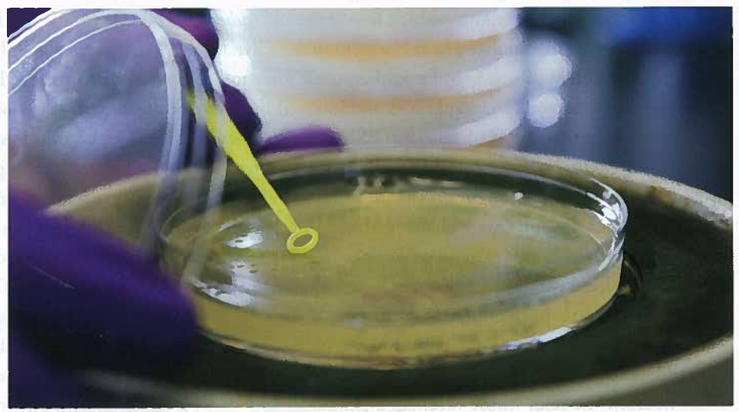
As Dr. Porcelli notes, the vast majority of people infected with TB bacteria do not develop active tuberculosis. "But the bacteria are never eradicated by the immune system," he says. "Rather, they're driven into a latent state and usually remain that way. If 100 people are infected, maybe only one or a few will reactivate. But if you have two billion infected worldwide—the current estimate—then it becomes an enormous problem, even if reactivation occurs in just a small percentage of those people."

Dr. Porcelli has been studying various aspects of TB ever since. He is currently focusing on how one subpopulation of lipid-recognizing T cells, called invariant Natural Killer T (iNKT) cells, respond to *M. tuberculosis* and how this response might be enhanced. He may have found an answer in a class of molecules called glycolipids.

Glycolipids are combinations of carbohydrates and lipids that are made by most living organisms. In recent years, scientists have shown that some types of naturally occurring glycolipids can selectively activate iNKT cells. And synthetic glycolipids called alpha-galactosylceramides activate iNKT cells even more strongly.

Now, in a study funded by the National Institute of Allergy and Infectious Diseases, Dr. Porcelli is synthesizing different alpha-galactosylceramide molecules and testing whether they can improve the immune response against *M. tuberculosis*. The results in mouse models have been encouraging, although he doesn't expect that his synthetic alpha-galactosylceramides will rev up the immune system sufficiently to eliminate TB infections. But the molecules could prove valuable as "adjuvants"—substances that increase the body's response to vaccines.

Individual TB bacteria form colonies on a special growth medium. Here, a technician picks out colonies for transfer to larger culture vessels so bacteria can multiply and be used in future studies.



"Sniff out what's interesting and go after it," says Dr. Porcelli. "You never know what you might find"

Dr. Porcelli is also trying to build a better TB vaccine. One project, a collaboration with Einstein scientists William R. Jacobs, Jr., Ph.D., and John Chan, M.D., has resulted in a new live attenuated vaccine built from *M. tuberculosis* itself. The researchers created the vaccine by deleting the bacterium's *sec2A* gene, which helps transport proteins across the cell membrane. The gene's presence also heightens *M. tuberculosis'* virulence by preventing infected host cells from displaying bacterial antigens to the immune system.

A first round of testing in mice showed that the *sec2A* vaccine achieved protection against TB that was significantly better than that obtained from BCG vaccination—exactly what the researchers were hoping for. But the microbes in the new vaccine retained some ability to multiply and infect the vaccinated animals. To counter this problem, the Einstein team deleted a second gene, *lysA*, that also influences *M. tuberculosis* virulence. Mouse studies involving this double mutant vaccine showed that a single immunization was significantly more effective than the BCG vaccine and also extremely safe. How it will fare in humans remains to be seen.

"BCG was developed almost a hundred years ago, and we still haven't improved upon it, which is pretty frustrating," says Dr. Porcelli. "Perhaps the human immune system lacks the basic weapons it needs to fight this bacterium. I don't believe that, although it's a viable hypothesis. I think the problem is that we still don't know very much about this organism. It has been 'studying' us for thousands of years. We're just beginning to catch up." E

"TB has been 'studying' us for thousands of years. We're just beginning to catch up"

A macrophage (gold) engulfs about 20 TB bacilli (green). Such bacilli not only survive ingestion by infection-fighting cells but actually multiply inside them.

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THE TWO-ARMED IMMUNE SYSTEM

The body responds to microbial invaders such as bacteria, parasites and viruses with two major lines of defense: cell-mediated immunity and humoral immunity.

In cell-mediated immunity, immune cells including T cells and phagocytes are aroused to attack the invading pathogen. In humoral immunity, the body makes B cells, which produce antibodies against certain microbial proteins known as antigens.

Both T cells and B cells can become memory cells, ensuring that the body will be ready to respond the next time it encounters the same antigen. The memory response can be triggered by infection as well as by vaccines, which contain microorganisms (or parts of microorganisms) that have been treated so that they provoke an immune response but not full-blown disease.