NEW TACTICS AGAINST TUBERCULOSIS

The pandemic is growing in many places, and strains resistant to all existing drugs are emerging. To fight back, biologists are applying a host of cutting-edge drug development strategies

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KEY CONCEPTS

- Tuberculosis is second only to HIV as the worldwide cause of death from infection, and the pandemic is growing in many places.
- TB is caused by a bacterium. Most cases are treatable, but strains resistant to first- and second-line drugs are on the rise.
- Conventional approaches to developing new antibiotics and vaccines against the disease have mostly failed.
- New tools are enabling scientists to study the TB-causing bacterium in greater detail, offering unprecedented insight into the interactions between pathogen and host. The results are exposing promising new targets for drug therapy.

_The Editors_

Bubonic plague, smallpox, polio, HIV—the timeline of history is punctuated with diseases that have shaped the social atmospheres of the eras, defined the scope of science and medicine, and stolen many great minds before their time. But there is one disease that seems to have stalked humanity far longer than any other: tuberculosis. Fossil evidence indicates that TB has haunted humans for more than half a million years. No one is exempt. It affects rich and poor, young and old, risk takers and the abstinent. Simply by coughing, spitting or even talking, an infected individual can spread the bacterium that causes the disease.

Today TB ranks second only to HIV among infectious killers worldwide, claiming nearly two million lives annually, even though existing drugs can actually cure most cases of the disease. The problem is that many people lack access to the medicines, and those who can obtain the drugs often fail to complete the lengthy treatment regimen.

Additionally, TB is evolving faster than our therapies are. In recent years, investigators have observed a worrying rise in the number of cases resistant to more than one of the first-line drugs used to treat the illness. Even more alarming, we have begun to see the emergence of strains that are resistant to every last one of the antibiotic defenses.

The disease is particularly devastating for the developing nations, where some 90 percent of cases and 98 percent of TB deaths occur. Beyond bringing untold suffering and sorrow there, TB harms entire economies. With 75 percent of cases arising in people between the ages of 15 and 54, TB will rob the world’s poorest countries of an estimated $1 trillion to $3 trillion over the next 10 years. Furthermore, the disease forces these struggling nations to divert precious resources from other important areas into health care. But the developed world would be mistaken to consider itself safe: although the incidence there is comparatively low, that situation could change if a highly resistant strain were to gain traction.

As bleak as this state of affairs is, we have reason to be hopeful. Cutting-edge biomolecular technologies are enabling researchers to study the complex interactions between the TB bacterium and the body in unprecedented detail, generating insights that are informing the development of novel diagnostic tests and drug therapies.
A Short-Lived Success
First identified by German physician Robert Koch in 1882, *Mycobacterium tuberculosis* (*Mtb*), the rod-shaped bacterium that causes tuberculosis, exists in both latent and active forms. In a latent infection, the immune system prevents the bacteria from multiplying, thus keeping them from disrupting tissues. Individuals with this form show no symptoms and are not contagious. Latent *Mtb* may persist for months, years or even decades without multiplying or making its host ill. Ninety percent of people infected with *Mtb* never develop active TB disease. But 10 percent of them do develop the active form, particularly those with weakened immune systems, such as young children and individuals who have HIV or are undergoing chemotherapy.

In people with active TB, the bacteria outpace the immune system, rapidly multiplying and spreading out to attack the organs. Primarily an aerobic bacterium, meaning it prefers environments rich in oxygen, *Mtb* has a special affinity for the lungs. Indeed, some 75 percent of patients with active TB exhibit the pulmonary variety of the disease. As the bacteria multiply, they destroy the lung tissue, commonly causing the host to develop such symptoms as a severe cough, chest pain and the coughing up of blood. But other organs are vulnerable, too. In fact, active TB can affect nearly every organ in the body. In children, TB can invade the cerebrospinal column, where it provokes a high fever with systemic shock—a condition known as meningitis. Left untreated, half of people with active TB die of it, most from lung destruction.

A century ago society had no way to combat TB, save for limiting its spread by sequestering affected individuals in sanatoriums. Back then TB, often called “consumption,” was widespread even in places that today have a relatively low incidence of the scourge, such as North America and western Europe. Scientists began to gain on the disease in 1921, when a vaccine made by French immunologists Albert Calmette and Camille Guérin, both at the Pasteur Institute in Paris, first entered into public use. (Initially believed to protect against both adult and childhood forms of the disease, the BCG vaccine, as it is known, was later shown through an extensive series of tests to confer consistent protection against only severe childhood forms.)

Twenty-two years later a team led by American microbiologist Selman Waksman developed...
streptomycin, which despite causing some side effects was the first effective therapy for TB. Waksman’s achievement opened the door for the creation in the 1950s of a rapid succession of antibiotics that compensated for streptomycin’s weaknesses.

Together these developments brought the era of sanatoriums to a close and significantly lowered the incidence of TB in countries that had the money and infrastructure to tackle the problem. By the 1970s many experts believed that TB had been almost completely eradicated. In reality, however, with international travel on the rise, the largest epidemics were just beginning. To make matters worse, those who would be hit hardest were those who could least afford it: residents of the poorest nations, who would soon also be facing a new and costly killer—HIV.

Today more than half a century after the debut of the first anti-TB drugs, the World Health Organization estimates that fully a third of the world’s population (more than two billion people) is infected with Mtb. On average, eight million of these carriers a year will develop active TB, and each will infect between 10 and 15 more individuals annually, maintaining the pandemic.

The picture becomes even more frightening when one considers the rising incidence of HIV. People who have latent TB and are HIV-positive are 30 to 50 times more likely than their HIV-negative counterparts to develop active TB, because HIV leaves their immune systems unable to keep TB in check. In fact, TB is the leading cause of death among HIV-positive individuals, claiming the lives of one out of every three worldwide and one out of every two in sub-Saharan Africa, where health care is especially hard to come by. Even if HIV-positive individuals have access to anti-TB drugs, their health will likely deteriorate because dangerous interactions between antiretroviral therapy and first-line TB drugs often force patients to suspend their antiretroviral therapy until the TB is under control.

The Latest Challenge

Perhaps the most disquieting aspect of the present pandemic, however, is the growing problem of the TB bacterium’s resistance to antibiotics. To understand how this predicament came to be, consider how TB is treated. The current treatment course, which was developed in the 1960s, is a demanding regimen consisting of four first-line drugs created in the 1950s and 1960s: isoniazid, ethambutol, pyrazinamide and rifampin. Patients who follow the regimen as directed take an average of 130 doses of the drugs, ideally under direct observation by a health care worker. This combination is extremely effective against active, drug-susceptible TB as long as patients are compliant and complete the entire six- to nine-month course.

Drug-resistant strains develop when patients do not complete the full protocol, whether because they start feeling better or because their drug supply is interrupted for some reason. Inconsistent use of antibiotics gives the bacteria time to evolve into a drug-resistant form. Once a drug-resistant strain has developed in one person, that individual can spread the resistant version to others. (For this reason, some authorities argue that it is better to not undergo treatment than to undergo incomplete treatment.)

According to the World Health Organization, nearly 5 percent of the roughly eight million new TB cases that occur every year involve strains of Mtb that are resistant to the two most commonly used drugs in the current first-line regimen: isoniazid and rifampin. Most cases of this so-called multidrug-resistant TB (MDR-TB) are treatable, but they require therapy for up to two years with second-line anti-TB drugs that produce severe side effects. Moreover, MDR-TB treatment can cost up to...
1,400 times more than regular treatment. Given that most MDR-TB occurs in impoverished countries, this expensive treatment is often not an option. Failure to properly diagnose MDR-TB, along with the high cost of treatment, means that only an estimated 2 percent of MDR-TB cases worldwide are being treated appropriately.

Worst of all, over the past few years health surveys have revealed an even more ominous threat, that of extensively drug-resistant TB (XDR-TB). This type, which made headlines in 2008 following an outbreak in KwaZulu-Natal, South Africa, is resistant to virtually all the highly effective drugs used in second-line therapy. Although XDR-TB is less common than MDR-TB, the possibility that XDR-TB will evolve and spread looms wherever second-line TB drugs are in use. World Health Organization records indicate that 49 countries had confirmed cases as of June 2008. That is a minimum figure, though, because very few countries have laboratories equipped to diagnose XDR-TB.

A Trickling Drug Pipeline
To say that scientists erred in assuming that the first-line drugs from the 1950s would be sufficient to combat TB is a profound understatement. But with the overwhelming majority of TB patients concentrated in some of the world’s poorest countries, large pharmaceutical companies have had little incentive since then to invest heavily in research and development for new drugs. And the prevailing wisdom among the greater pharmaceutical conglomerates is still that the cost of drug development—$115 million to $240 million and seven to 10 years per drug—far outweighs the potential global market for such products.

Thanks to government programs and private philanthropic organizations such as the Bill and Melinda Gates Foundation, however, many efforts are under way to create TB antibiotics to both treat drug-resistant cases and reduce the time that it takes to treat normal TB cases. As a result, a few promising agents are currently in early clinical trials. One such agent, known as SQ109, inhibits cell wall synthesis. It recently completed phase I (safety) clinical trials. Another drug candidate is PA-824, a compound whose ability to attack Mtb in both its actively dividing stage and its slow-growing one has generated hopes that the drug could significantly reduce the time needed to treat the disease. PA-824 is in phase II clinical trials, which look at efficacy.

Unfortunately, the odds are against these candidates: historically, fewer than 10 percent of antibiotics that enter early clinical trials garner approval—a success rate that derives in large part from the outmoded logic used to discover these drugs. Fifteen years ago developing new antibiotics was mostly a matter of following a simple formula: identify enzymes that are essential to bacteria survival and that do not have any counterparts in humans; screen libraries of compounds for potent inhibitors of these enzymes; chemically synthesize derivatives of those inhibitors; then optimize the compounds for druglike properties, such as the ability to get from the stomach to the bloodstream. Yet even the large pharmaceutical companies, masters of developing medicines to treat nearly any disease, have been spectacularly unsuccessful in producing new antibiotics using this approach.

For its part, the TB battleground is littered with the corpses of drug candidates that failed. Many of these compounds were highly specific and potent inhibitors of key TB enzymes. In some cases, although they effectively foiled isolated enzymes, they flopped when tested on whole bacterial cells. In others, the compounds thwarted whole bacteria in test tubes (in vitro) but missed their mark when tested in infected animals. TB offers perhaps the most extreme example of the troubling disconnect between the in vitro and in vivo effects of antibiotics. Most of the time investigators have absolutely no idea why drug candidates fail. The crux of
Tuberculosis, caused by the bacterium *Mtb*, occurs in both latent and active forms. People can become infected by breathing in even just a few *Mtb* bacteria released into the air when those with active TB cough, spit or talk. *Mtb* causes coughing, the most familiar symptom, because it accumulates abundantly in the lungs, but it can harm other organs as well (diagram).

* Mtb tends to concentrate in the air sacs, or alveoli, of the lungs because it prefers environments rich in oxygen. In most people, the immune system is able to keep bacterial replication in check, dispatching defensive cells known as macrophages to the site of infection, where they form a shell around the bacteria. But in 10 percent of infected individuals, *Mtb* breaks down the shell, after which it can begin to multiply.

Unfettered by the immune system, the bacteria destroy the tissue of the lungs; some may also make their way into the bloodstream and infect other parts of the body, including the brain, kidneys and bone. Eventually affected organs may sustain so much damage they cease to function, and the host dies.

The problem is that bacteria are autonomous life-forms, selected throughout evolution for their ability to adapt and respond to external threats. Like modern aircraft, they have all manner of redundancies, bypasses, fail-safes and emergency backup systems. As Jeff Goldblum’s character in *Jurassic Park* puts it, life finds a way. Until we truly appreciate the complexities of how TB interacts with humans, new drugs against it will remain elusive. The good news is that we are making progress on that front.

**Insights from “Omics”**

A key turning point in our TB education came in 1998 with the sequencing of the DNA code “letters” in the *Mtb* genome—a project in which one of us (Barry) participated. That sequence, and those of related organisms, has yielded a trove of insights. Perhaps most importantly, the results showed that of all the enzymes and chemical reactions that are required for TB to survive in a human, we were considering only a third of them in our in vitro (test tube) tests. We learned, for instance, that *Mtb* devotes a huge amount of its genome to coding for proteins that synthesize and degrade lipids, suggesting that some of those proteins might be worth considering as drug targets. Analysis of the TB genome also hinted that, contrary to conventional wisdom, the bacterium is perfectly capable of living in the absence of air—a suggestion now verified. Under such anaerobic conditions, *Mtb*’s metabolism slows down, making it intrinsically less sensitive to existing antibiotics. Targeting the metabolic elements that remain active under these circumstances is one of the most promising strategies for shortening treatment time.

Translating the information we have gleaned from the genome into discoveries that can help save the lives of people who contract TB has neither been simple nor straightforward. But recently researchers have used those data to make significant advances in diagnostic tests for the disease. Diagnosis can be complicated by the effects of the childhood vaccine, which is given to more than half of all infants born around the world. The vaccine contains a strain of *Mtb* that has lost its virulence yet is still able to induce a child’s immune system to react against the TB bacterium. Vexingly, though, the predominant test for TB cannot distinguish between immune responses elicited by virulent *Mtb* and the vaccine form. Hence, the test results for someone
who is infected look exactly like the results for someone who has been vaccinated.

While the Mtb genome was undergoing sequencing, scientists in Seattle discovered that a large stretch of DNA was missing from the bacterial strain used in the vaccine. Shortly thereafter, independent research teams at the Pasteur Institute, the Albert Einstein College of Medicine and the University of Washington showed that the missing genes were essential to virulence. The deleted region in the vaccine strain thus offered investigators a strategy for improving the specificity of the test. A test that searched only for an immune response directed against the virulence factors absent from the vaccine strain, the researchers reasoned, should be able to distinguish infected individuals from those who had been vaccinated. In fact, just such a test was developed and approved by the U.S. Food and Drug Administration in 2005, and many recent studies have confirmed its accuracy. Unfortunately, so far the cost of the test is high, which restricts its use to the First World.

The Mtb genome is not the only new source of data able to provide insight into the TB bacterium’s potential vulnerabilities. Scientists can now study all kinds of cell components and processes—from all the proteins in a cell (a discipline known as proteomics) to the amount of messenger RNA (the templates from which proteins are made) made from every gene (“transcriptomics”) to the intermediate and final products of cell metabolism (“metabolomics”). These fields are still in their infancy, but already they have borne fruit. Last November, Barry co-authored a paper in Science reporting that when TB was treated with PA-824, the bacterial transcriptome reacted exactly as if it had just been poisoned with potassium cyanide. This finding was a vital clue that in metabolizing the drug, Mtb releases nitric oxide, a defensive molecule normally made by immune cells in the human body. Armed with this knowledge, we and others are now synthesizing compounds that stimulate the release of larger amounts of nitric oxide than are elicited by PA-824 and so should be even more potent against Mtb.

Complementing those approaches, structural genomics seeks to uncover the three-dimensional structure of every protein in Mtb—work that can both help identify the still-mysterious functions of many Mtb proteins and aid the design and synthesis of drugs targeting particular sites on critical proteins. So promising is this line of

[worldwide resistance map]

Tuberculosis occurs in virtually every country in the world, although it is most widespread in developing nations. The incidence of TB caused by strains of Mtb resistant to two or more of the first-line drugs for the disease—so-called multidrug-resistant TB (MDR-TB)—has been rising as a result of improper use of antibiotics. Worse still is extensively drug-resistant TB (XDR-TB)—a largely untreatable form identified in 2006; as of June 2008, 49 countries had confirmed cases. Sadly, that figure most likely underestimates XDR-TB’s prevalence.
from 17 countries is focusing its efforts entirely on the structural genomics of Mtb. Thus far the consortium has helped determine the structure of about 10 percent of the organism's proteins.

Another "omics" branch worth noting is chemical genomics, a very recently established field of research that effectively reverses the standard process of drug discovery. Instead of starting with a protein of known function and looking for a compound that inhibits its activity, investigators begin with a compound known to have a desirable trait—such as an ability to inhibit Mtb reproduction in cell cultures—and work backward to identify the microbial enzyme impaired by the substance. The compounds can be anything from molecules synthesized in a chemistry lab to products isolated from plants, microbes and even animals. The starting chemical in this case serves strictly to reveal vulnerable enzymes or biological processes, which scientists may then identify as targets for drug development.

What makes this approach so appealing is that it allows us to harness the power of natural selection in our quest to thwart Mtb. Before Mtb and other mycobacteria found humans to be such appealing hosts, they occupied environmental niches where they had to compete with countless other bacteria for food in a constant arms race. Bacterial ecosystems have therefore undergone multiple rounds of natural selection, and in most cases other bacteria have evolved ways of keeping the mycobacteria in check, as is evident from the diversity of bacteria types in these ecosystems. If researchers could tap into the amazing reservoir of weapons that these competitor bacteria have evolved—applying modern omics tools to identify the defensive molecules, screen them for their anti-TB potential and pinpoint their molecular targets in Mtb—we could well uncover entirely new classes of drugs. We could then select those agents that knock out the pathogen's whole system, as opposed to just a single process for which Mtb likely has a workaround.

**A Model Bacterium**

To reap the full benefits of the omics revolution, we need information technology tools capable of making sense of the vast data sets generated by omics experiments. In fact, the development of such tools has become a discipline unto itself, called bioinformatics. And only with these tools can researchers hope to clear another obstacle to drug development: that posed by so-called emergent properties—behaviors of biological systems that cannot be predicted from the basic biochemical properties of their components.

To borrow an example from neuroscience, consciousness is believed to be an emergent property of brain biochemistry. In the case of in vitro Mtb, one emergent property is a tendency of the bacteria to form "cords"—serpentine arrays...
that have a ropelike appearance; these cords result from complex interactions among molecules on the bacterial surface, and their development is not predictable from the properties of the molecules involved. Correspondingly, in a human host, the interactions among such surface molecules and the cells of the immune system result in the formation of a granuloma—a large aggregate of host cells and bacteria that is very difficult for drugs to penetrate. The granuloma, too, is an emergent property of the interaction between Mtb and its host.

With the aid of bioinformatics, we hope to ascertain how all 4,000 of Mtb’s genes, their corresponding proteins and the bacterium’s metabolic by-products react when Mtb is treated with a new drug in vitro. Moreover, in the past 10 years we have begun piecing together exactly how the bacterium operates inside of TB patients, as opposed to in vitro. The ultimate goal is to replicate Mtb in silico—that is, produce a computer simulation of the bacterium that behaves just like the real thing does in the body. The significance of such an achievement cannot be overstated, because it will enable investigators to accurately predict which bacterial components make the best drug targets and which drug candidates will likely hit those targets most effectively.

To achieve this objective, scientists will need to trace in exquisite detail all of the organism’s biochemical pathways (series of reactions) and identify more of the emergent properties that arise from the operation of these pathways. The task is enormous: we still do not know what perhaps a third of Mtb’s proteins do in the first place, never mind what their associated pathways are or what emergent properties they spawn. But based on the current rate of progress, we are confident that within the next 20 years we will see a complete in silico bacterium that acts exactly like its counterpart growing in a test tube in the lab—and maybe even in a human being.

Preventing TB infection in the first place is, of course, better than treating people after they have become sick. To that end, efforts to create a vaccine that confers better protection against the disease than does the BCG vaccine are under way. Some developers are trying to improve the existing vaccine; others are attempting to make entirely new ones. But for the moment, the work is mostly doomed to trial and error because we do not understand why the current vaccine does not work nor how to predict what will work without testing candidates in humans.

In other diseases for which vaccines are available, surviving an initial infection provides immunity to future infection. In TB, however, initial infection does not offer any such protection. A vaccine that is based simply on an attenuated version of TB therefore will not work. And whereas drug development would be greatly accelerated by the development of an in silico bacterium alone, enhanced vaccine development would require both an in silico bacterium and an in silico human to be successful. Such an arrangement would allow us to systematically explore the effects on humans of altering the bacterium.

In his book The Tipping Point, Malcolm Gladwell defines said point as “the level at which the momentum for change becomes unstoppable.” Never has the need for better diagnostic tests, drug therapies and vaccines against TB been greater. Much work remains to be done, but with the genomes of both Homo sapiens and Mycobacterium tuberculosis decoded and with an unprecedented amount of brainpower now trained on the problem, the momentum for change truly is unstoppable.

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IN THE TRENCHES

New drugs will be critical for combating TB, but public health officials cannot afford to wait until they are available. In the meantime, programs such as the World Health Organization’s Stop TB Partnership are working to stem the pandemic by improving quality control at testing facilities, enhancing patient supervision and support, ensuring drug supply and educating the public about care, among other tactics. The program aims to reduce the number of deaths from TB by more than half by 2015.

MORE TO EXPLORE


Tuberculosis information from the World Health Organization is available at www.who.int/tb/en

www.SciAm.com