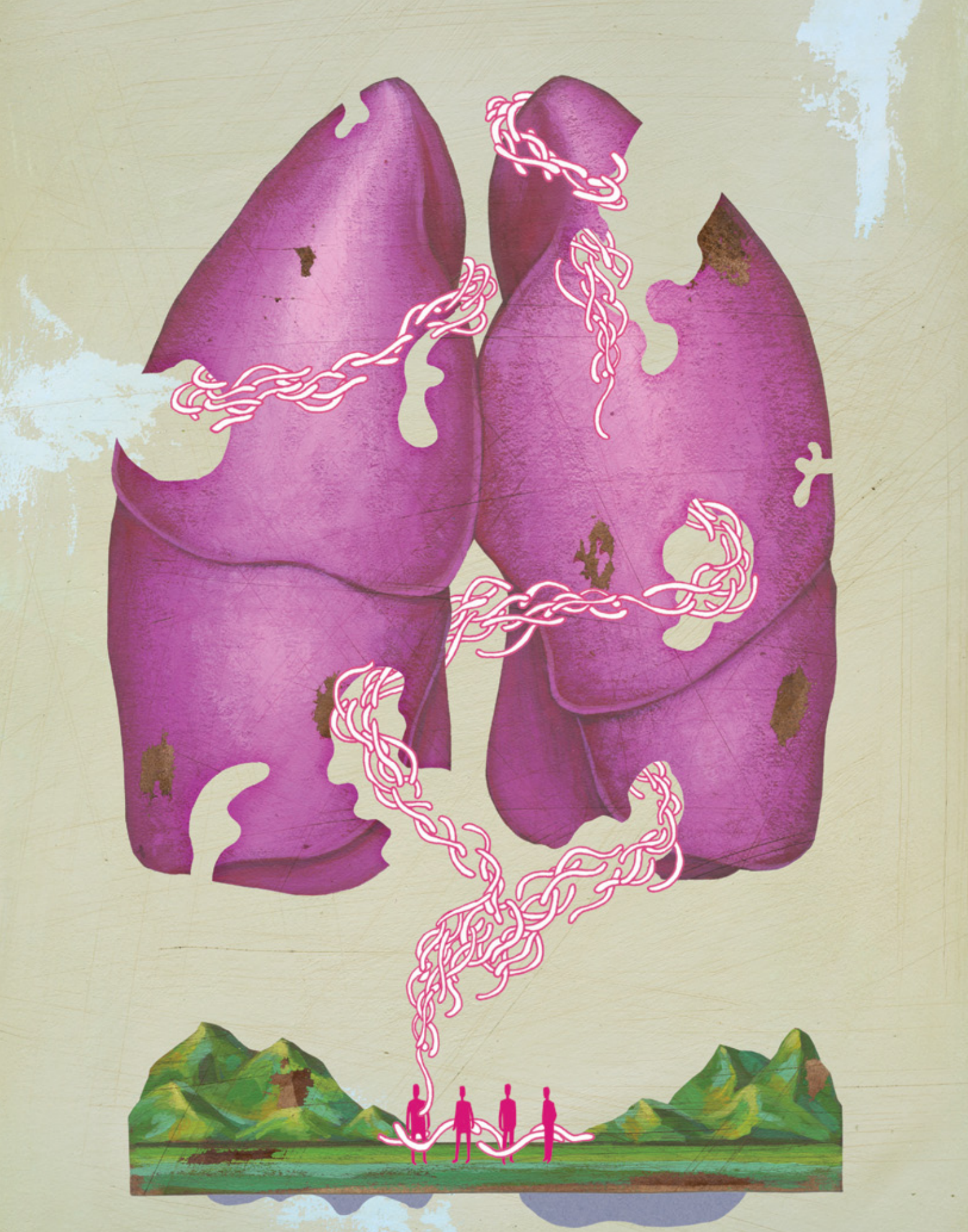

GLOBAL HEALTH

The Diabolical Genius of an Ancient Scourge

Tuberculosis seems to be evolving in unexpected ways that outsmart humans

By Sally Lehrman

TODAY MOST PEOPLE IN THE RICHER PARTS OF THE WORLD THINK of tuberculosis, if they think of it at all, as a ghost of history. Throughout ancient times the tenacious bacterial infection consumed the bodies of untold millions, rich and poor, filling their lungs with bloody sputum. As TB spread in the centuries that followed, it continued to attack across economic and class lines, affecting both the famous and the obscure. Among its better-known victims: poet Manuel Bandeira, writers Emily and Anne Brontë, and sculptor Frédéric-Auguste Bartholdi, who designed the Statue of Liberty. By the early 20th century humanity had begun fighting back with public health campaigns, improved living standards, and eventually antibiotics and a modestly effective vaccine. Although in 2011 TB sickened nearly nine million people, killing 1.4 million of them, mostly in the poorer regions of the globe, the mortality rate has nonetheless fallen by more than a third since 1990. Things are looking up—or so it may seem.

Illustration by Daniel Chang

New genetic research, however, suggests that the bacterium responsible for TB could be poised to emerge stronger and more deadly than ever before—and not just because some strains have become resistant to treatment with the standard set of antibiotics. A small but increasingly influential group of investigators believes that the microbe, *Mycobacterium tuberculosis,* may have evolved along an unexpected and particularly dangerous path. The scientists have discovered that TB can be divided into seven families of genetically related strains, at least one of which is surprisingly virulent, prone to drug resistance and especially well suited to spreading disease in our increasingly interconnected, densely populated world.

At the same time, researchers worry that current approaches to treatment and the sole, partially effective vaccine may actually be helping the bacterium to become more intractable. Clinicians have long known that incomplete treatment can produce antibiotic-resistant TB strains. Yet they are now realizing that even successful interventions can be problematic if they are better at weeding out the milder, slower-growing groups of TB microbes. This divergent effect would allow the more aggressive, faster-spreading bacterial families to establish a stronger foothold.

What is more, efforts to develop new therapies and diagnostic tests may be doomed to fail if the strains being targeted are not the ones that are spreading around the planet. If these fears are realized, TB rates could one day begin rising again globally, and the disease could become harder to treat and spread more widely among populations that have so far been relatively free of the scourge.

Still, there is room for hope. The genetic work provides some insight into how to fight back against the more worrisome groups of TB germs. "Maybe the goal shouldn't be to eradicate the disease," suggests Clifton E. Barry III, chief of tuberculosis research at the National Institute of Allergy and Infectious Diseases. Instead of trying to eliminate all disease-causing TB microbes, he and others propose, the aim should perhaps be to favor bacteria that are milder and more likely to stay in a dormant state. Engineering such a successful standoff is, of course, a difficult and complex proposition.

A MYSTERIOUS OUTBREAK

THE LATEST FINDINGS grew out of research into another dismaying surprise that TB unleashed on public health experts, starting in 1986. That year officials in New York City were completely caught off guard by an aggressive outbreak of multidrug-resistant TB that took about a decade and hundreds of millions of dollars to bring under control—mostly by rigorously tracking patients with active illness and making sure that they finished six to nine months of treatment with a combination of antibiotics. (In some cases, it takes two years to kill all the bacteria present.)

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At the time, experts had become so confident in their ability to control TB that most programs meant to detect tuberculosis cases had been shut down and funding for research had slowed to a trickle. The National Institutes of Health reduced its spending on TB to a meager $300,000 in 1985, and the academics who studied TB could, according to one researcher, practically squeeze themselves into a single minivan. In New York City—which for more than a century had been the site of both the illness's worst ravages and the greatest public health strides against TB—only eight treatment clinics were still open by the late 1980s.

Within a few years the steady decline in cases stopped, and without apparent warning, the trend line reversed itself. Standard anti-TB medicines could no longer predictably tame the infection, even with a diligent patient who stuck to the arduous regimen.

Alarmed public health authorities considered all the possible explanations. Many of the new cases occurred in recent immigrants and some HIV patients, which made sense. About a third of the global population harbors a latent TB infection until something—such as stress or another illness—reactivates the bugs, leading both the bacteria and the body's own immune response to attack lung tissue, setting transmission to other individuals into motion. Immigrants were arriving from Southeast Asia, East Asia and Mexico, where TB rates were 10 to 30 times higher than in the U.S. The high incidence in HIV patients in the mid-1980s also seemed logical because those individuals often had compromised immune systems, which might allow a latent infection to become active.

Yet those standard explanations for the outbreak did not fit all the facts. This time around, tuberculosis was spreading faster through New York City's vulnerable populations than anyone had seen for at least a couple of generations, and people were dying at a much higher rate than normal. Something else had to be driving TB's reemergence, which quickly began claiming lives in Florida, Hawaii, Texas and California as well.

NEW INSIGHT

THE ANSWER, at least in part, turned out to be activity by a formerly unrecognized group of TB bacteria that spread more readily and are more deadly than the classic bug, which tends to proliferate slowly and enters a long quiescent phase after the

IN BRIEF

More than one million men, women and children around the globe die of tuberculosis every year, and about a third of the world's population harbors a latent infection.

A growing number of studies suggest that TB may be evolving into a new bug that is far more deadly, spreads more quickly and is more likely to become resistant to treatment with antibiotics.

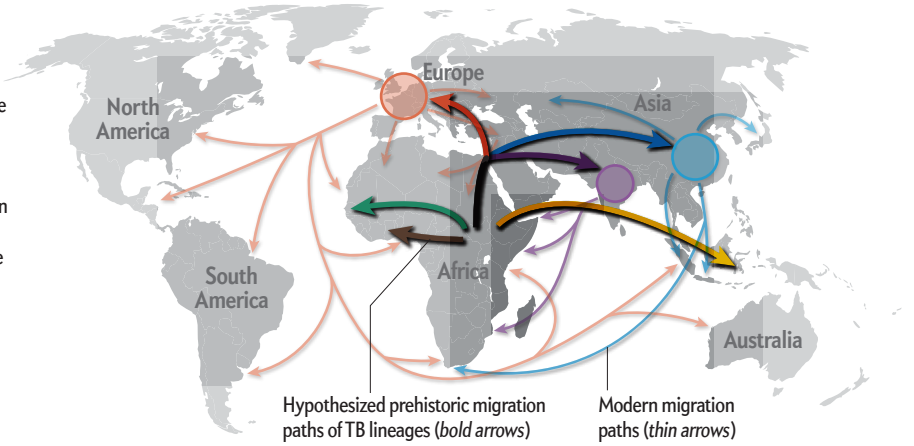
Designers of new treatments should take these latest findings into account if they do not want to make matters worse. Changing the host environment with improved housing, for example, may also prove key.

How TB Conquered the World

Scientists have long assumed that tuberculosis emerged around 10,000 years ago, when humans began domesticating cattle. Recent genetic analysis suggests, however, that the TB germ may in fact predate the first major human migration 60,000 to 70,000 years ago. As TB-infected people spread across the world, the pathogen eventually evolved into at least seven families, or lineages.

Out of Africa and Back Again

The two oldest families of TB germs began in West Africa, where they are still found. Four more lineages took hold around the Indian Ocean and in India, East Asia and Europe. Further migration and colonization led to a greater spread. Still unclear: why the slave trade did not establish the West African lineages in the Americas.



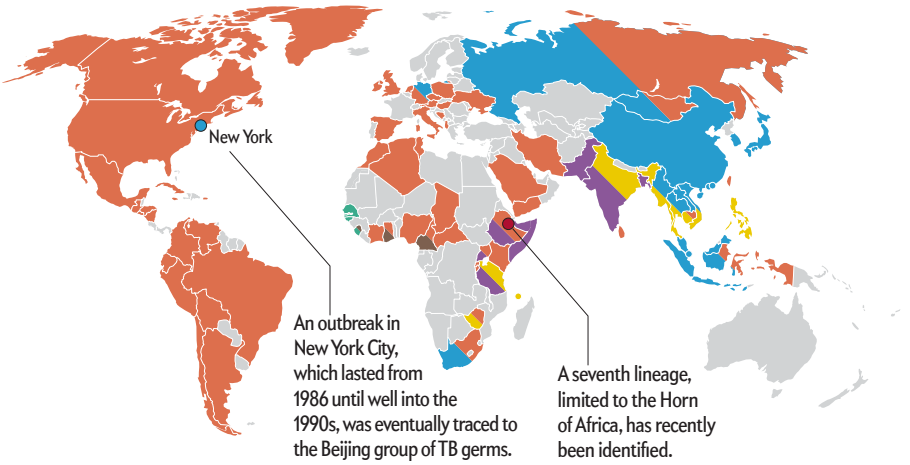
Major TB Lineages

Ancient
- West African 1
- West African 2
- Indo-Oceanic
- Hypothesized common ancestor of modern lineages

Modern
- Euro-American
- East African–Indian
- East Asian

Current Distribution

Different families of TB germs spread at different rates. Africa is the only continent where all the TB lineages that have so far been identified can still be found. A particularly dangerous member of the East Asian family of TB germs, known as the Beijing group, is now threatening the globe.



initial infection, including in untreated cases. The body, mounting an immune response, walls off the bacteria into a cavity, and the two begin an uneasy truce that can last for decades.

Today researchers call the newly identified collection of TB microbes the Beijing group (because the greatest concentration of cases was later found in the Chinese capital). Eventually they learned that it is a subset of one of six large families of TB germs. (A seventh family, found so far only in the Horn of Africa, has been reported in the past six months.) Until the early 1990s, no one had realized that *M. tuberculosis* even had multiple families.

The first clues that TB strains fall into distinct groups came in 1991 in San Francisco, during an outbreak in a homeless shelter for people with HIV. Peter Small, now a senior program officer at the Bill & Melinda Gates Foundation, was then a resident at San Francisco General Hospital, where he worked with Philip Hopewell, a prominent tuberculosis expert. Small had just learned how to track the spread of individual TB strains using certain patterns that appeared in their DNA—a powerful new molecular biology technique that was then being developed. While public health servants took up the time-honored task of contacting everyone who had come into contact with an infected person, Small was given the job of identifying and tracing the TB germs involved.

The results were frightening: of the 14 people from the shelter who had fallen ill over four months, Small found that 11 shared the same strain of tuberculosis, which was identified by its unique DNA fingerprint, a pattern of code letters found only in that strain. Having the same strain meant that the illness in those 11 individuals stemmed from recent transmission of the infection, as opposed to reactivation of latent infections (which would have yielded dissimilar genetic profiles). Furthermore, the progression from initial infection to full-blown disease and transmission to another person was lightning-fast.

SOURCE: "HIGH FUNCTIONAL DIVERSITY IN *MYCOBACTERIUM TUBERCULOSIS* DRIVEN BY GENETIC DRIFT AND HUMAN DEMOGRAPHY," BY RUTH HERSHBERG ET AL., IN *PLOS BIOLOGY*, VOL. 6, NO. 12: DECEMBER 16, 2008 (*top*); "VARIABLE HOST-PATHOGEN COMPATIBILITY IN *MYCOBACTERIUM TUBERCULOSIS*," BY SEBASTIEN GAGNEUX ET AL., IN *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA*, VOL. 103, NO. 8: FEBRUARY 21, 2006 (*bottom*)

Maps by XNR Productions

"It was a huge wake-up call," Small says. Investigators had expected to find reactivated disease in individuals with compromised immune systems, not new infections. And they were stunned by how quickly the bacterium spread from one person to the next and by how rapidly the illness progressed. HIV and TB seemed to be acting synergistically in their attacks on people's immune systems. A germ that raced through its latent stages faster and that was more infectious would be especially tough to bring under control and to keep contained.

When the team broadened its study to include immigrants, they found exactly the pattern that they had expected, which provided no comfort. This time genetic tests showed that the illness stemmed, as anticipated, mostly from latent infections that had been reactivated.

Not all the TB strains that the researchers found spread at the same rate, however, which was odd because they believed at the time that all strains behaved more or less alike. Small and his colleagues would find the TB fingerprint of one patient all over the city, whereas that of another, very similar patient would not show up in anyone else. "You couldn't help but think, 'Well, maybe the bacteria differ,' which was a pretty radical thought at the time," Small says.

In the complex dialogue between the tuberculosis organism and the human body, some strains excel at inhibiting the immune system, whereas other strains boost it.

Their findings had important implications for public health; clinicians needed to step up their efforts to reduce transmission and to ensure that patients completed their treatments. They also challenged researchers to rethink their understanding of the organism itself, including when tuberculosis might have first affected humans. If all TB strains belonged to one big family that caused illness in the same manner (as had long been assumed), chances were that *M. tuberculosis* had originated fairly recently, perhaps 10,000 years ago. If, on the other hand, separate families of TB microbes had evolved and were spreading at different rates, then the organism had probably been around much longer than anyone had suspected, giving it plenty of time to diversify. Indeed, in 2005 researchers at the Pasteur Institute in Paris performed a genetic analysis that suggested *M. tuberculosis* could have evolved from an ancestor species as early as three million years ago.

STARTLING EVIDENCE

THE SAN FRANCISCO BAY AREA turned out to be an ideal location to test the hypothesis that *M. tuberculosis* could be divided into distinct families of microbes associated with specific geographical regions. With immigrants from Africa, Latin America, Eastern Europe and multiple regions in Asia, it is, in many respects, a microcosm of the world. In the early 2000s a group of investigators—many of whom had worked with Small and Hopewell during the TB outbreak in San Francisco—began studying samples from various TB patients and comparing the molecular markers in their bacterial genomes.

Using 875 strains collected between 1991 and 2001 from individuals representing 80 countries, the group identified fragments of DNA present in some strains but not in others. Based on these differences, the scientists found that the strains sorted into six main families that had apparently originated in different regions of the world and, it also seemed, were still infecting people who had recently lived in those places. There were three ancient ones, including two found only in West Africa and another that arose in Africa, then migrated with humans along the Indian Ocean more than 60,000 years ago. Three more modern lineages developed in western Europe (traveling to the Americas at the end of the 19th century), northern India and East Asia (the Beijing group turned out to be a prominent member of this family). Africa was the only location that seemed to play host to all six lineages, although the Euro-American family was widespread, and the Beijing strains were rapidly gaining a foothold around the globe.

Working with population geneticist Marcus Feldman and others at Stanford University, Sebastian Gagneux, then at the Institute for Systems Biology in Seattle, traced each lineage's ancestral life story. By comparing the DNA sequences of 89 critical genes (most of which were vital to the bacterium's continued survival), Gagneux and his colleagues could estimate different lineages' ages and geographical movements. These so-called housekeeping genes are under tremendous evolutionary pressure to stay the same; any changes are more likely to harm rather than help the bug. So the more closely matched the strains, the more closely related they would be, and the most genetically diverse groups would belong to the oldest families.

The oldest TB lineages from Africa, the researchers theorized, may have taken root in small, scattered hunter-gatherer groups. At that time, limited opportunities for transmission may have produced TB's characteristic latency. It could, for instance, infect a child, wait a generation and reactivate in time to infect new family members. As ancient humans began to migrate over land, the group proposed, the organism tagged along, and the Indo-Oceanic lineage developed, taking advantage of an increasing population. Later migrations and population expansions provided fertile ground for the three more modern lineages to emerge and adapt to their hosts. As humans traveled, traded, crammed into crowded cities, went to war, and died, tuberculosis went along for the ride, causing increasingly frequent and more severe illness.

The genetic clustering among the lineages provided the evidence that the mycobacterium had evolved along with its hosts. Gagneux, emphasizing that it was all a careful guess, proposed an Out of and Back to Africa hypothesis. Modern lineages had emerged along early human migration routes out of Africa, he suggested, then had more recently gone back to the continent and out again. The Euro-American family of strains, for instance, followed colonization to Africa, Asia and the Middle East. The East Asian lineage moved to South Africa via Southeast Asian slaves in the 17th and 18th centuries, with another wave following via Chinese gold miners.

The diversification of the bacterial families and their dissemination around the globe pointed to a complex coevolution between host and pathogen that is probably still under way. Whenever people jammed into overcrowded living spaces, the more aggressive TB strains with shorter latency periods spread rapidly. Meanwhile the older, West African and Indo-Oceanic lineages, which tended to thrive in less populated areas, caused an illness that progressed more slowly. "If there are very few hosts, it doesn't pay to be very virulent, because you kill all your hosts, then you die out together with all your hosts," says Gagneux, who now heads up tuberculosis research at the Swiss Tropical and Public Health Institute in Basel. One two-year study in the Gambia seems to support this idea: patients exposed to the modern TB strains were nearly three times as likely to progress to active disease. In fact, the more aggressive strains of TB have begun overtaking the oldest pair of lineages even in Africa.

NOW WHAT?

ALL THE DATA GENERATED since the 1990s have consistently marked the Beijing group of strains as particularly worrisome. It seems to spread more easily and to cause more severe disease, and it may even be especially adept at becoming resistant to antibiotic drugs. In 1998 investigators determined that the aggressive strains that had spurred the outbreaks in New York City in the 1980s and 1990s fell within this group as well.

Just as important in fueling TB's continuing ravages around the globe are the environmental conditions under which people live. Small moved to India in 2011, where he still resides, to learn about the realities of living with TB in one of its most devastating breeding grounds. TB germs do not spread in a vacuum, he notes. People with tuberculosis might also be malnourished or alcoholic or might avoid taking medication. Not just HIV but also diabetes seems to interact synergistically with the organism to manipulate the immune response in ways that facilitate transmission and activation. Social conditions such as crowded housing, poor air quality, hunger and stigma tend to make matters worse.

The interplay of bacteria and human environments is worth noting, Small says. Investigators suspect that some strains of TB, for example, tend to provoke a brisk immune response, leading to the quick development of cavities in the lung and rapid progression from latency to illness. Other strains tend to suppress the immune system, making their home in different organs. In the complex dialogue between host and pathogen, Small says, "some of these strains seem to be really good at dialing down the immune system and others at dialing it up."

In a close examination of genetic molecules across a variety of strains, Small and Gagneux found that the bug did not follow the evolutionary pathway of most human pathogens. Instead of changing over time, the DNA that gave rise to the germ's outer proteins (the part that is recognized and targeted by the body's immune system) stayed the same. More typically, disease-causing bacteria are forced to change their protein coverings or risk being eliminated from the human population within a few generations. This bizarre finding has serious implications for some of the new vaccines that are now being developed against tuberculosis. Vaccines, by definition, are designed to boost the body's immune response to quash an infection. Yet for TB, this enhancement could perversely enhance transmission. A family of bacteria that has evolved to boost the immune response might be helped, not hurt, by a vaccine that has further activated the immune system of people who were inoculated.

"Again it's a bit complicated," Small explains. Once inside the body, the TB germ actually does not do very much. It is the body's own attempts to rid itself of the infection that causes the most damage. For example, the white blood cells of the immune system create the cavities in the lungs where TB gets walled off. "Thus, augmenting the host response could be helping the bug, not the host," he says. "This is just a theory, as a strong response could also prevent the bug from getting a foothold in the first place. But if true, it has important implications."

Evolutionary biologist Paul W. Ewald of the University of Louisville backs up Small's concerns. The vaccine in use today, which primarily protects children in high-risk areas from developing such severe complications as TB meningitis, is about 90 years old and has been given to about one billion people. Ewald suggests that the inoculation, which is based on a weakened strain of a closely related bacterium that infects cattle, may have inadvertently encouraged more deadly strains of *M. tuberculosis* to flourish. "It's dawning on people that this is a sophisticated organism that's evolving with humans," Ewald says.

This interaction implies that learning how to direct evolution of TB through the use of standard public health measures and more sophisticated therapies might help defeat it. Better housing that decreases crowding and enhances air ventilation, for example, might favor less powerful strains of TB. But improving living conditions for the one billion people who live in the world's slums is a lot harder than handing out pills. (On the other hand, the passage and enforcement of a law in 1901 that mandated greater access to air and light in tenement buildings helped to decrease TB infection rates in New York City in the years before antibiotics.) Gagneux, for his part, also foresees the need to bring together immunologists, ecologists, evolutionary biologists, population geneticists and social scientists to tackle all aspects of TB's ability to transmit itself, cause disease and adapt to different environments. Such cross-disciplinary partnerships often sound better on paper than they work in practice, he admits: "But ultimately, I think that's what we need."

Gagneux would like researchers who are developing new diagnostic tools, treatments and vaccines to at least consider testing them against various strains from different parts of the world. Right now most are checked solely against strains that were first grown in laboratories more than 60 years ago and may no longer be relevant. With some lineages potentially being naturally resistant to new drugs or predisposed to evade diagnostic tests, ignoring TB's family tree could prove to be a death sentence for millions more people around the world. SA

MORE TO EXPLORE

Worldwide Occurrence of Beijing/W Strains of *Mycobacterium tuberculosis*: A Systematic Review. Judith R. Glynn et al. in *Emerging Infectious Diseases*, Vol. 8, No. 8, pages 843–849; August 2002. wwwnc.cdc.gov/eid/article/8/8/02-0002_article.htm

Host-Pathogen Coevolution in Human Tuberculosis. Sebastien Gagneux in *Philosophical Transactions of the Royal Society B*, Vol. 367, No. 1590, pages 850–859; March 19, 2012. http://rstb.royalsocietypublishing.org/content/367/1590/850.long

SCIENTIFIC AMERICAN ONLINE

View a slide show about the effort to fight tuberculosis that spans a century and several continents at ScientificAmerican.com/jul2013/tuberculosis