WHO/N. OTTO

Hong Kong and other regions in South-East Asia are trying to get to grips with a resurgence in tuberculosis.

LATENCY

A sleeping giant

Most people infected with Mycobacterium tuberculosis *never get the disease, but predicting who will is turning out to be a complex problem.*

BY COURTNEY HUMPHRIES

Among the elderly population in Hong Kong, a spate of tuberculosis (TB) is worrying public-health officials. But these cases are not the result of recent infection; they signal the emergence of disease from an infection picked up decades ago. "Almost all recent cases in the elderly have arisen through reactivation of long-term latent infections rather than recent new infections," says Benjamin Cowling, an infectious disease epidemiologist at the University of Hong Kong's School of Public Health in China.

Improved standards of living and public-health campaigns in most of the industrialized world dramatically reduced rates of TB during the first half of the twentieth century. But, unlike in the West, Hong Kong, Japan and Korea only began to get to grips with TB after the Second World War. So people who were first infected as children back in the 1940s and 50s — and who have shown no sign of TB for over 50 years — are succumbing to the disease as their immune systems weaken. "Given a high prevalence of latent TB in the elderly, and currently no strategies to stop reactivation," Cowling says, "it is difficult to foresee major changes in TB incidence in the coming years."

By current estimates, two billion people are infected with *Mycobacterium tuberculosis* worldwide, but only 10% will develop active disease in their lifetimes. The rest have what are called latent infections: they are not sick and will not spread the infection to others[1]. The risk of developing disease is roughly 5% in the first 18 months, and 5% over the rest of one's life[2].

This lurking threat is motivating researchers to study TB latency. Are there differences in the bacteria or in the host immune response that determine who gets the disease? Should everyone with latent infection be treated, or are there biomarkers or molecular signatures that could distinguish those who will develop disease?

TRICKY CUSTOMER

Latency is not a well-defined biological condition; it describes anyone who tests positive for TB and does not have clinical symptoms, says David Sherman, a biochemist at the Seattle Biomedical Research Institute in Washington. The assumption has been that *M. tuberculosis* remains viable in people with latent infections, not causing disease but maintaining its potential to do so.

This ability to lie dormant may be an evolutionary strategy of the bacterium. Douglas Young, a microbiologist at the MRC National Institute for Medical Research in London, points out that TB first appeared when early modern humans lived in small communities. "If TB was as virulent then as it is now, you would expect those populations to be wiped out," he says. "There's a big advantage to being latent, letting the population reproduce and then infecting the next generation." The microbe may have only acquired more virulent traits as human settlements grew and it was possible to flourish despite proving fatal to some of its hosts.

But the distinction between latent and active disease may not be as clear-cut as once thought. Ongoing studies of patients with latent TB suggest that although some may have completely controlled the infection, others might have undetected subclinical disease. "Latency encompasses a grab bag of individuals at different points in the spectrum of disease," says Clifton Barry, chief of the Tuberculosis Research Section at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland.

This emerging view of latent infection is supported by research on the pathology of *M. tuberculosis* infection in animals. JoAnne Flynn, a microbiologist at the University of Pittsburgh in Pennsylvania, worked for years to create a

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model of latency in mice. But her breakthrough came about a decade ago when she was working with cynomolgus macaque monkeys, and noticed that about half of the TB-infected animals did not go on to develop disease.

Flynn saw a surprising heterogeneity in lung pathology among individual monkeys — and even within the same individual. She has focused her sights on pockets of inflammation called granulomas: collections of mycobacteria, macrophages and other immune cells, sometimes surrounding dead tissue. Granulomas can vary in size, composition and degree of organization, and are thought to represent an attempt to contain the infection[3]. "It turns out latent monkeys can have a number of granulomas, even one or two that look active," she says. The pathology of the lungs is less severe that those with active infections, but the distinction is not absolute.

SUMMING THE PARTS

Using positron-emission tomography (PET) and computed tomography (CT) imaging, Flynn is able to observe individual lesions in the lung over time, and then perform molecular analyses in more depth. "Each granuloma is its own little world," she says, and whereas some of them are able to control the infection, some are not. Far from hiding or going dormant, the bacterium seems to be involved in an active, dynamic battle in which the immune system fights to keep the disease in check across various individual sites. If that is true, says Sherman, "the distinction between latent TB and active TB becomes an arbitrary one".

The notion that latency represents the sum of its parts would make it difficult to find systemic biomarkers of TB progression. "It suggests that looking at the global immune response is unlikely to be able to predict who is going to do well and who is not," Flynn says. However, studying individual lesions could illuminate the factors that allow host and microbe to coexist for long periods of time, and why the balance sometimes fails.

Barry has been using the same imaging method to study lung lesions in people with active TB, and now in two groups in South Africa and South Korea with latent infections. He has already observed lesions in some people with latent infections and the study "definitely supports the idea that latency in people is incredibly heterogeneous". But it is too early to say whether the extent of the lesions predicts whether people get the disease, as it seems to in monkeys.

To figure out why TB lesions can vary so much, researchers are looking at how the heterogeneity within populations of mycobacteria might influence the microenvironment of the granulomas, and their interaction with the host's immune system. Sarah Fortune, a microbiologist at the Harvard School of Public Health in Boston, Massachusetts, has been working with Flynn to develop molecular tools to analyse the variability of bacteria in monkeys with TB.

Fortune and her team have created bacteria engineered with genetic sequence tags that act as molecular barcodes: one sequence that identifies each strain, and a randomized sequence unique to each bacterium. It allows them to track the fate of different strains and of individual bacteria and their progeny. They have also taken advantage of the fact that when TB bacteria die, they remain in granulomas. Researchers can count the genomes of dead and living bacteria in these bacterial graveyards, helping them to understand the course of the infection. Fortune believes that even bacteria that are genetically identical may differ in gene expression. "There's probably a lot of individuality in the bacterial population that hasn't been appreciated," she says.

For public-health efforts to tackle the reactivation of latent infections, the challenge is first to identify, among the vast numbers of people worldwide with latent infections, those most in need of treatment. "The key issue in managing latent TB is diagnosing people who are at risk of progressing to disease," says Barry.

In 2010, Anne O'Garra, an immunologist at the MRC National Institute for Medical Research, reported progress on this front. Her research showed that the transcriptional profiles of people with active versus latent TB differed. Such profiling could lead to better diagnostic tests to distinguish latent from active infection, or a prognostic tool to determine which people with latent infections will go on to get active disease or to track treatment success in diseased people. Robert Wilkinson, an infectious disease specialist at Imperial College London and the University of Cape Town who collaborates with O'Garra's team, says that they are now testing the approach in larger and more complex groups of patients, including people with HIV and other diseases. "The crucial clinical question is to tell [the profile] apart from diseases that might mimic TB," he says, pointing out that it is not yet clear whether this information can be divined from a transcriptional profile.

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TREAT IT RIGHT

Latent infections can be successfully treated, but if doctors treated everyone then ten people would be getting drugs for every one who would have developed disease, says Richard Menzies, director of the Respiratory Division at the Montreal Chest Institute in Canada.

Consequently, Western governments have made recommendations to screen for and treat latent TB based on risk factors. For example, updated guidelines in 2011 for England and Wales recommend screening all recent migrants from countries where TB is widespread, and suggest that treatment should be considered in people with latent infection who are immunocompromised (such as through HIV infection).

In poor countries where TB infection is endemic and resources are limited, that level of treatment is not feasible, nor is it the priority when so many people with active infections are transmitting disease. But even in developed countries, both doctors and patients are reluctant to initiate treatment in the absence of disease. "There's widespread acceptance of drug treatment for conditions that predispose people to illness, such as hypertension or high cholesterol," says Menzies, "but somehow the notion is you don't have to treat latent TB infection," even though the odds of preventing later disease and of experiencing side effects are comparable.

Ensuring patients complete their treatments, a challenge in patients with active TB, is even more problematic for patients who are not even sick: a long medication regimen for a disease they might never get is a tough sell. Menzies believes that until treatment regimens are available that have a negligible risk of side effects, doctors will continue to be reluctant to prescribe treatment and patients unwilling to accept it.

Developing drugs specifically for latent infection is difficult, as at present drug makers are required to show the efficacy of a drug against replicating bacteria or active disease in mice, but Sherman says that there is a lot of interest among researchers in targeting latency. "Many people now believe that the key to shortening therapy for active disease is to find agents that work on latent disease," says Sherman, because bacteria may be living in a variety of conditions and metabolic states that overlap between the two conditions, and these states may make them more or less susceptible to different drugs.

Isoniazid targets bacteria as they replicate, but drugs developed to exploit other bacterial functions could be more effective at treating latent infections. For instance, researchers at the Institute Pasteur Korea in Seongnam-si have found a compound that targets ATP synthesis in *M. tuberculosis*, a process that is slow in bacteria that are not replicating, but which they still need to survive. Such a drug could benefit both active and latent disease[4].

The situation in Hong Kong illustrates how leaving latent infections untreated is a stumbling block to achieving very low incidence rates. "It's guaranteed that we can never break the cycle if we only treat the active cases," says Barry. Cowling agrees. "Latent TB is going to be a problem for many years to come," he says. And he believes that what is happening now in more developed areas such as East Asia is a harbinger of future problems in less developed countries that are still grappling with active disease. ■

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