Breaking the Code for the Tuberculosis Invasion

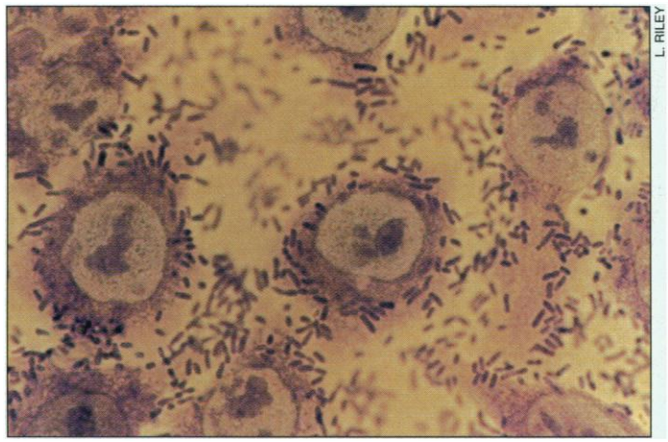
Escherichia coli is ordinarily harmless. Not only is it used in the lab as a model organism to express genes, but it's also present in enormous quantities in everyone's intestines. Now, however, a team led by molecular epidemiologist Lee Riley of Cornell University Medical College in New York has created a recombinant *E. coli* that mimics some of the traits that make *Mycobacterium tuberculosis*, the agent of tuberculosis (TB), such a deadly pathogen.

And that's just its virtue, as this research development—safely contained in a secure P3 facility—represents an important breakthrough in TB research: Riley's team, which reports its results on page 1454, has for the first time cloned a DNA fragment that allows the pathogen to invade and survive inside human cells. It's providing researchers with their first insight into the genetics of TB infection, and they're delighted at the prospect. "It's very important," says bacterial geneticist Neil Stoker of the London School of Hygiene and Tropical Medicine. "There is so little known about the molecular basis of virulence" in *M. tuberculosis*.

Shortly after *M. tuberculosis* enters the body, it invades cells called macrophages that travel through the bloodstream ingesting foreign microorganisms. The TB pathogen isn't engulfed and destroyed, however. Instead, it actively penetrates macrophages, where it can sit for years before embarking on an orgy of destruction in the victim's lungs that leads to illness and death. If investigators can determine how the organism is able to enter macrophages safely, they might just be able to develop ways to nip TB infections in the bud.

To find the genes responsible for the bacterium's ability to invade macrophages, Riley's team inserted random DNA fragments from the *M. tuberculosis* genome into *E. coli*. Normally *E. coli* doesn't enter human cells, but Riley's team eventually found a 1535-base fragment of *M. tuberculosis* DNA that, when inserted into *E. coli*, produced a bacterium adept at invading cultured human epithelial cells called HeLa cells. Unlike macrophages, HeLa cells don't actively engulf particles—which means that the recombinant *E. coli* must have initiated the entry itself.

Buoyed by this success, the Cornell researchers set about finding the location of the "invasion gene" on the fragment. "We started chopping off one end of it," Riley explains. It was a process of elimination: By inserting these smaller fragments into *E. coli*, the researchers found that the factor responsible for cell invasion is coded by a region somewhere in the initial 850 bases of the larger fragment: If the researchers deleted part of this region, the recombinant *E. coli* no longer invaded HeLa cells.



L. RILEY

Tuberculosis mimic. Recombinant *E. coli* bacteria, with TB pathogen DNA added to their genome, have invaded these HeLa cells.

The remaining 685 bases seem to help *M. tuberculosis* survive inside macrophages, in a hostile environment rich in the lysosomal enzymes that break down engulfed pathogens. Recombinant *E. coli* containing the entire 1535-base fragment survived well in cultured macrophages, Riley's team found, whereas recombinants containing only the smaller DNA fragment responsible for cell invasion were quickly destroyed.

The scientists don't yet know whether the two DNA fragments, which influence the separate abilities to invade human cells and then to survive in the hostile intracellular environment, encode different parts of the same protein molecule or instead contain separate genes. But the Cornell team has already discovered one protein that seems to be encoded by the 1535-base fragment. This 52 kiloDalton polypeptide, alien to *E. coli*, is produced by the invasive recombinant strain. Riley expects to purify the protein within a year, raise antibodies against it, and then use an antibody-based stain to work out where the protein is expressed. This could provide clues to the protein's function. If it interacts with a receptor carried by macrophages, for instance, it should show up on the surface of *M. tuberculosis* cells.

Another possibility is that the protein is secreted into the so-called electron transparent zone (ETZ). This is a clear region surrounding the TB pathogen that's been seen on electron micrographs of infected macrophages. Some researchers believe the ETZ prevents lysosomal enzymes from reaching the bacterium. The recombinant *E. coli* does seem to produce an ETZ of its own, but Riley says it's too soon to view this as a solid link between the zone and pathogen survival, as the apparent ETZ found around the invasive *E. coli* could be an artifact. "It would be too good to be true," he says.

Of course, the major weakness with the Cornell team's research is that all their work has so far been done in a model organism, and not the TB pathogen itself. Researchers would like to move from the recombinant *E. coli* to *M. tuberculosis* to learn whether knocking out the Cornell team's DNA fragment seriously impairs the bacterium's ability to enter and survive in human cells. Unfortunately, however, there are some obstacles that need to be surmounted before these knockout experiments can be done. In other bacteria, genes can easily be removed, disrupted by inserting some additional DNA, and then replaced. But in *M. tuberculosis*, researchers haven't yet discovered how to reintroduce the disrupted genes at the correct position in the genome. "We need to develop this ability in tuberculosis genetics," says molecular geneticist Bill Jacobs of New York's Albert Einstein College of Medicine.

Nevertheless, Riley is optimistic that a complete molecular understanding of his recombinant *E. coli*'s ability to infect human cells will eventually lead to clinical advances in the fight against TB, such as vaccines designed to prevent infection of macrophages by *M. tuberculosis*. A vaccine based solely on a protein encoded by the 1535-base DNA fragment is unlikely to work, Riley predicts, because the pathogen probably employs more than one cell entry mechanism. But it may be possible, he says, to design multicomponent vaccines based in part on the protein that he's now trying to purify.

The technical difficulties inherent in working with the TB organism mean it may be years before this happens. But, says Stoker, "it's only by doing these sort of experiments that we're going to know where the weaknesses in the bacterium's life cycle are." And the Cornell team's results mean that TB researchers finally have their feet on the first rung of the ladder.

–Peter Aldhous