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A Chink in the Armor of the TB Bacillus

One of the world's deadliest microbes, *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis (TB), has tipped off researchers to a potential chink in its armor. Researchers from the Howard Hughes Medical Institute (HHMI) have identified a lipid molecule that must be produced by *M. tuberculosis* if the bacterium is to infect the lungs of mice.

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— William R. Jacobs Jr.

"The unique lipids of *M. tuberculosis* likely play key roles in making the bacterium the world's most successful pathogen having infected more than one in three people on the planet," said William Jacobs, an HHMI investigator at Albert Einstein College of Medicine. "This work is the first to establish definitively that exported lipids are required for the bacteria to grow in the lungs."

"What really distinguishes tuberculosis as a pathogen is that it passes from person to person by aerosol infection, but it first has to invade the lungs to propagate," said Jacobs. "If we understood how TB propagates in the lungs, we'd have a much better idea of how to combat the infection."

The rapid emergence of drug-resistant strains of *M. tuberculosis* has placed a premium on the need to develop new ways to stop TB infection. One route, pursued by Jacobs and his colleagues, is to understand at the molecular level how *M. tuberculosis* invades the lungs and thrives in this otherwise hostile environment. In time, observations from such studies could pave the way for improved vaccines and drugs to prevent TB, which kills more people each year than AIDS or malaria.

In studies published in the November 4, 1999, issue of the journal *Nature*, Jacobs and colleagues Jeffery Cox and Bing Chen of Albert Einstein College

of Medicine and Michael McNeil of Colorado State University inserted bits of gene-disrupting DNA called transposons at random locations in the genome of *M. tuberculosis*. Each transposon created a mutation that carried a signature sequence of DNA that could be easily identified later hence, the technique's name "signature-tagged mutagenesis."

The scientists then infected mice with the mutant strains of bacteria, each of which contained a unique DNA tag. Three weeks later, they harvested the bacteria from the lungs of the mice and looked for strains of bacteria that failed to thrive. They quickly zeroed in on three mutant strains of *M. tuberculosis* that did not flourish in the lungs of mice despite being able to grow in the liver and spleen. Looking closely at the mutations, they noticed that all three strains contained mutations in a region of the tuberculosis genome where genes responsible for making the lipid phthiocerol dimycocerosate (PDIM), a component of the bacterial cell wall, were known to be located.

"At that point, though, we had shown only that the mutants failed to grow in the lungs," said Jacobs. "But when we grew the bacteria on agar, we found that they formed quite distinctive colonies that indicated defective cell wall biosynthesis."

Wild-type TB bacilli form flat and corded colonies on agar, but the mutant colonies looked strikingly different. "They looked like the Pompidou Center in Paris, with the pipes running all over the outside of the building," explained Jacobs.

Further experiments revealed that two of the mutant strains of bacteria could not synthesize PDIM. The third mutant produced normal levels of the lipid. Additional studies revealed, however, that this mutant contained a defective gene called *mmpL7* and could not transport PDIM outside of the bacterial cell, where the lipid apparently aids the infection process.

"This finding established that PDIM is required for virulence, and has to be exported from the cell," said Jacobs. "What's particularly exciting about the discovery of the *mmpL7* mutant is that the gene is a member of a family of 12 highly homologous genes. We postulate that all of these genes are involved in exporting lipids that are known to be unique to this bacillus. We believe that we will be able to determine the function of all these lipids and to gain significantly greater understanding of the pathogenesis of the tuberculosis bacillus," said Jacobs.

Jacobs speculated that the lipids might play a key role in the bacillus's ability to overcome macrophages, immune system cells that prowl the lungs engulfing and shredding invading bacteria.

"The challenge will be to figure out whether these lipids are an offensive or defensive weapon," said Jacobs. "Are these lipids protecting the bacillus from some killing function of the macrophage? Or, are the bacteria using these lipids to launch an offensive strike on the macrophage?"