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Enzyme Offers Target to Attack Drug-Resistant Tuberculosis

Researchers have discovered an enzyme that allows the tuberculosis (TB) bacterium to metabolize nutrients and persist for months while shielding itself from immune-system attack by hiding inside macrophages.

According to the researchers, the enzyme, called isocitrate lyase (ICL), offers a prime target for drugs to kill persistent strains of TB bacteria that can evade antibiotics and cause chronic infection. A drug that targets ICL, says Howard Hughes Medical Institute investigator William R. Jacobs, Jr., may be able to reduce the time required to treat TB infection. Jacobs and his colleagues have also determined the crystal structure of ICL bound to inhibitors of the enzyme giving them a head start in developing such drug treatments.

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Collaborating research teams led by Jacobs, who is at Albert Einstein College of Medicine, Cornell researcher David Russell and Texas A researcher James Sacchettini announced their findings in articles in the August 17, 2000, issue of the journal *Nature* and in the August 2000 *Nature Structural Biology*.

"Tuberculosis has an amazing ability to grow exponentially until the immune system kicks in," said Jacobs. "Once the immune system engages, tuberculosis stops growing, but it can persist despite immune-system attack and antibiotic drug treatment. This extraordinary persistence is why tuberculosis claims more human lives each year than any other bacterial pathogen. And it is why we have concentrated on understanding the factors underlying persistence."

The researchers began their search for persistence factors when they saw studies by other scientists showing that a persistent form of TB seemed to depend solely on lipids for its sources of energy and carbon. Dependence on lipids implied that this strain of TB relies on the glyoxylate shunt, a metabolic pathway that aids in the production of four-carbon compounds for use in making vitamins and the recycling of amino acids, which are the basic

building blocks of proteins.

"The enzymes that constitute the glyoxylate shunt pathway are attractive drug targets because that pathway is not found in mammals," explained Jacobs. "So, if you make a drug that inhibits the shunt, it will not affect human cells."

Lead author of the *Nature* paper John McKinney, a postdoctoral fellow in the Jacobs laboratory, proceeded to develop mutant strains of TB in which ICL, a key glyoxylate shunt enzyme, was deleted. "It was very stunning that during the first three weeks the mutant bacteria grew as well as the wild-type bacteria," said Jacobs. "Later, the mutants failed to persist and kill the mice, as do the wild type bacteria."

Meanwhile, Russell and his colleagues, working separately from Jacobs' team, found that ICL was the most highly upregulated protein in persistent TB bacteria that had been isolated from macrophages. Macrophages are predatory immune system cells that prowl the lungs, engulfing and digesting invading bacteria. Normally in a resting state, macrophages are activated by the host's immune response that develops subsequent to bacterial invasion. The TB bacillus, however, possesses the uncanny ability to enter macrophages and to use them as refuges while in the persistent state.

"Given that it's generally accepted that macrophages are the seat of the TB infection, the finding of ICL upregulation gave us an inroad to understanding the metabolic pathways required for intracellular survival," said Russell. "So, we cloned and expressed the enzyme and began to characterize it biochemically."

When the two research groups learned of each other's findings, they began a collaboration to explore the enzyme's role. Their collaborative studies revealed that the mutant TB that lacked ICL grew adequately in resting macrophages but were seriously impaired in activated macrophages.

"These findings lead us to believe that if we could inactivate ICL, we would have the ability to kill persistent TB," said Jacobs. "Right now, the course of chemotherapy for TB is six months because of the inability to kill persistent bacteria, so such drugs could make an enormous difference."

A third collaborating group, led by Sacchettini, used x-ray crystallography to obtain a detailed structure of the ICL protein. In particular, the scientists determined structures of the protein bound to inhibitor compounds identified by the Jacobs laboratory. Lead authors of that paper are Vivek Sharma and Sujata Sharma at Texas A According to Sacchettini, the enzyme-inhibitor structure, reported in the *Nature Structural Biology* article, offers important clues to designing drugs to attack persistent TB bacteria.

"This structure provides us with a template for future drug design experiments," he said. "We've learned that when the enzyme binds to a substrate or inhibitor, it undergoes large conformational changes that close its active site. Now that we know this, we can design compounds that trap the protein in this closed conformational state and inhibit the enzyme." In

collaboration with Glaxo Wellcome, the researchers have already launched a search for such compounds.

ICL is the second enzyme identified by Jacobs and his colleagues as crucial to the persistent state of the TB bacillus. In April 2000, Jacobs and his colleagues reported that an enzyme called a cyclopropanase was crucial to the bacteria's ability to form the ropelike colonies characteristic of the virulent form. According to Jacobs, both ICL and cyclopropanase offer important insights into the persistent state of TB infection, as well as new avenues for improved therapy for TB.