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## Sweet New Ways to Fight Tuberculosis

Antibiotics have been used since the 1940s to cure tuberculosis. But the bacterium that causes the disease,

*Mycobacterium tuberculosis*

, keeps evolving to dodge the drugs that are thrown at it, and existing treatments are becoming less effective. Now, Howard Hughes Medical Institute (HHMI) scientists have found several new ways to kill

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*M. tuberculosis*

, which could lead to the development of alternative drugs.

*M. tuberculosis* is transmitted through the air and infects about one-third of the world's population. It often remains latent, and causes no harm to 90 percent of those infected. But more than nine million people each year develop active cases of TB, which typically causes weight loss, night sweats, and lung damage. Nearly two million people die each year from what should be a curable disease, either because they are not properly diagnosed, do not take the right medications, or have drug-resistant strains that defy treatment.

"We found two sweet new ways to combat tuberculosis by targeting enzymes in pathways that metabolize carbohydrates," said William R. Jacobs, Jr., an HHMI investigator at the Albert Einstein College of Medicine, whose team published their findings in the March 21, 2010, issue of *Nature Chemical Biology*. "Now we need drugs that inhibit these enzymes."

Extremely drug resistant tuberculosis (XDR-TB), first identified in KwaZulu-Natal, South Africa, can evade up to 10 different drugs. The

pathogen tends to develop this drug resistance while in its dormant state, and so Jacobs set out to better understand that stage. “We wanted to know what *Mycobacteria* eat when they are in the persistent state. That led us to look at glucan synthesis,” said first author Rainer Kalscheuer, a postdoctoral researcher in the Jacobs lab.

*M. tuberculosis* uses glucan as an energy storage compound. Enzymologists Karl Syson and Stephen Bornemann at the John Innes Center in Norwich, UK, collaborated with Jacobs’ team and found that two enzymes, TreS and Pep2, work together to transform a starting material – the sugar trehalose – into an intermediary sugar molecule called maltose 1-phosphate. An enzyme called GlgE, which Jacob’s team had shown was essential for survival in *M. tuberculosis*, converts maltose 1-phosphate to glucan. Their experiments demonstrated that although the bacteria needed maltose 1-phosphate to make glucan, if too much of it accumulates within cells, it becomes toxic.

In experiments with *Mycobacteria smegmatis*, which are closely related to *M. tuberculosis*, the scientists prompted the cells to use the trehalose-glucan pathway by feeding them trehalose, and found that under those conditions the bacteria needed GlgE to survive. An absence of the GlgE enzyme caused maltose 1-phosphate to build up until the cells, in effect, poisoned themselves. When they conducted similar experiments with *M. tuberculosis*, they found that the pathogen routinely relies on the trehalose-glucan pathway, and that inactivating GlgE caused an accumulation of maltose 1-phosphate that killed the bacteria as effectively as either of the two front-line TB drugs, isoniazid and rifampicin.

Once they had demonstrated this “self-poisoning” effect, Jacobs’ team tested how stalling the trehalose-glucan pathway at its intermediary step affected the pathogen’s ability to infect mice. Bacteria without the GlgE enzyme died quickly in infected animals, they found. “Inactivating a key gene in this pathway, *gIgE*, kills TB in a different way than we have ever seen before,” Jacobs' said. “We were completely surprised because nobody ever knew that bacteria could convert trehalose to glucan.”

“We essentially produced a metabolic disease in *Mycobacterium* that kills it,” Jacobs said, likening the toxicity to naturally occurring genetic diseases in humans that interfere with the conversion of nutrients and cause the build-up of toxic intermediates. These diseases are often detected during postnatal screening and many are treatable by diet, enzyme replacement, or other strategies.

The trehalose-to-glucan pathway, however, is not found in mammalian or other eukaryotic cells, nor in the beneficial bacteria that live in the gut. That makes the pathway an attractive drug target, Jacobs says, because inhibiting it would not affect human cells or digestion.

The researchers then asked if interfering with one of the two previously known glucan biosynthesis pathways would also kill *M. tuberculosis*. That revealed a second death mechanism: simultaneously targeting two components of a pathway producing glucan derivatives called methylglucose lipopolysaccharides. They found that *M. tuberculosis* can survive without a gene in the pathway called *Rv3032*, and also when the TreS enzyme was inhibited. But they found that inhibiting TreS in bacteria without *Rv3032* was lethal.

“We don’t yet know what essential function TreS and *Rv3032* jointly perform, but if we could inhibit both of them simultaneously, we could have an effective new tuberculosis drug,” said Jacobs. “If we could combine that drug with a GlgE inhibitor, both of these new death mechanisms would work in synergy, enhancing the killing effect on *Mtb* cells and reducing the probability of tuberculosis becoming drug resistant.”