New findings on drug tolerance in TB suggest ideas for shorter cures

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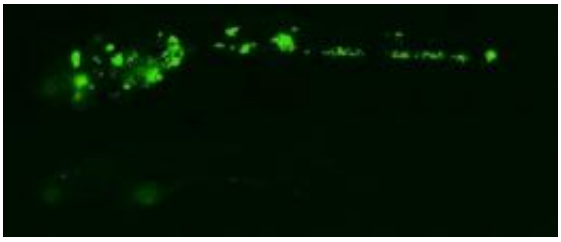

The top zebrafish is heavily infected with TB bacteria, which are green fluorescent. The bottom fish has been treated with isoniazid for five days. While most of the infection is cleared, making the bottom fish barely visible, you still can see the residual, antibiotic-tolerant bacteria. Credit: Lalitha Ramakrishnan Lab

New findings on how tuberculosis (TB) bacteria develop multi-drug tolerance point to ways TB infections might be cured more quickly.

The study will be published April 1 in the journal *Cell*. The results identify both a mechanism and a potential therapy for drug tolerance that is induced in the TB bacteria by the host cells they infect.

Currently, TB treatment requires a complex, long-term curative regimen of at least six months, explained the senior author of the study, Dr. Lalita Ramakrishnan, University of Washington (UW) professor of medicine, microbiology and immunology. Her lab conducted the study in collaboration with Dr. Paul Edelstein, of the Department of Pathology

and Laboratory Medicine at the University of Pennsylvania.

Many months of TB treatment are needed because the bacteria become tolerant to TB drugs. In the first couple days of treatment the bacteria die rapidly, but the death rate then slows to a crawl as the bacteria become resistant to killing. This tolerance to antibiotics occurs despite the absence of genetic mutations for drug resistance.

The authors noted that adhering to six months of drug treatment is difficult, particularly in areas of the world where TB is prevalent.

"Breaks in treatment can lead to relapses that perpetuate the TB epidemic and also fuel the development of genetic resistance to treatment," Edelstein said.

An urgent goal of scientists is to overcome drug tolerance, yet most of the drugs in development will not shorten the lengthy treatment. This failure results from a poor understanding of the mechanism of TB tolerance.

"Drug tolerance," Ramakrishnan said, "has been largely attributed to TB bacteria that are dormant in the body and not reproducing. These postulated dormant bacteria are thought to be unaffected by the administered antibiotics that are most effective against rapidly growing organisms"

In this latest study, the researchers draw another picture of what might be happening in TB. They described the existence of multi-drug tolerant organisms that form within days of infection in zebrafish, an animal model for studying TB. However, they were surprised to see this bacterial population actively growing and reproducing inside of host macrophages – "big eater" white blood cells that engulf germs and debris in the body. In fact, the drug-tolerant bacteria not only thrive within

these disease-fighting clean-up cells, but also co-opt them to carry the transiently drug resistant TB infection to other parts of the body. There they help promote its continuation inside of granulomas – the nodules in the lung characteristic of TB.

To understand how growing bacteria might be tolerant to drugs, the authors turned to studying the human TB bacillus in human macrophages. They found that upon infecting macrophages, the bacteria deploy a structure called an efflux pump. The authors found that these pumps are essential for the TB bacteria to grow within macrophages and are turned on possibly to get rid of host antimicrobial substances targeted to penetrate the bacteria and destroy them.

Paradoxically it is the infected macrophage itself that stimulates the efflux pumps in the bacteria. The TB cell efflux pumps can also flush away medications, a mechanism, the researchers say, which may be contributing to multi-drug tolerance.

"Thus the TB drugs get caught in the crossfire in this pitched battle between bacterium and host," Ramakrishnan said.

Certain inexpensive medications are already available that can inhibit these bacterial pumps and thereby reduce multi-drug tolerance, the researchers noted. One of these is the calcium channel blocker verapamil, which is used to treat high blood pressure, angina, and some heart rhythm problems.

The researchers suggest that adding this currently approved drug to TB therapy, or working to develop drugs that more specifically block the bacterial efflux pumps in TB organisms, might reduce multi-drug tolerance. If so, efflux pump inhibitors might be able to shorten the duration of curative treatment for TB.

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