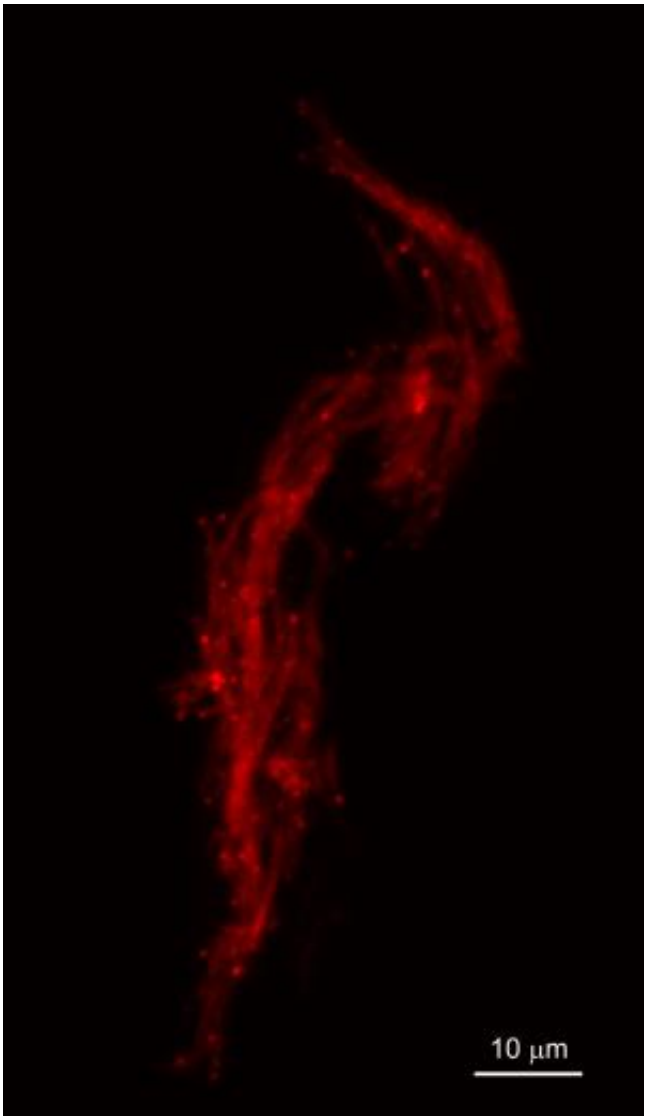
Cell-destroyer that fights and promotes TB reveals what's behind its split identity

April 11 2013



Exuberant mycobacterial growth showing chains of fluorescent microbes in an infected zebrafish larva which had an excess of tumor necrosis factor. Credit: Francisco Jose Roca Soler

Tumor necrosis factor—normally an infection-fighting substance produced by the body—can actually heighten susceptibility to tuberculosis if its levels are too high. University of Washington TB researchers unravel this conundrum in a report this week in *Cell*.

Their study shows how excess production of this disease-cell destroyer at first acts as a TB germ killer. But later the opposite occurs: too much tumor necrosis factor encourages TB pathogens to multiply in the body.

In addition to figuring out some reasons behind this back-pedaling, the scientists learned that certain combinations of drugs already available for other conditions can curtail the shift from anti-TB to pro-TB.

The drug combination revealed in this study, the authors noted, "has the potential to revert some cases of hypersusceptibility to hyperresistance."

The scientists were Francisco Jose Roca Soler, of the UW Department of Microbiology, and Lalita Ramakrishnan, UW professor of microbiology, medicine and immunology. A recipient of the National Institutes of Health Director's Pioneer Award, Ramakrishnan is recognized for her work on how the TB pathogen and its hosts' cells interact to cause disease.

These studies are conducted in zebrafish, an animal model for tuberculosis. The fish's embryos and small fry are transparent. Researchers can see through their skin to observe their organs, tissues and cells and the internal appearance of some infections, for example, the bacterial cording of TB.

Roca and Ramakrishnan explained that TB had traditionally been thought of as a disease of failed immunity. However, more recent studies

from their lab and other labs, both in zebrafish and in humans, have suggested that it also can result from too strong of a defensive inflammatory response.

"While tumor necrosis factor is a critical host defense against tuberculosis," Roca and Ramakrishnan noted, "an excess of this factor is also implicated in the development of the disease in zebrafish and in humans."

Variations in a specific location of the zebrafish genome can cause either too much or too little tumor necrosis factor to be produced, depending on the type of variation. In either case, deficiency or overabundance, zebrafish become prone to tuberculosis.

In both cases the scavenger cells, or macrophages, that are trying to clear away the TB pathogens by ingesting them, die and burst open. They are like torn vacuum cleaner bags spilling their dirty contents.

When the TB bacteria escape the confines of the scavenger cells, "they grow exuberantly in the extracellular environment," Roca and Ramakrishnan said.

Researchers needed to work out the differences between TB susceptibility caused by too high or too low tumor necrosis factors because the distinction is vital to treatment decisions. Only patients whose genetics made them launch a pro-inflammatory response, benefited from steroid treatment, previous studies have shown. Steroids can increase the chance of death among TB patients with a weak inflammatory response.

In the present study, Roca and Ramakrishnan elucidated the molecular pathways by which too much tumor necrosis factor at first rapidly promotes macrophages to go after TB bacteria, and then turns around

and forces the hard-working macrophages to die and expel their captives.

They found that both the microbiocidal activity, and the death of the macrophages, resulted from upping the production of reactive oxygen species by the mitochondria inside the macrophages. Mitochondria are the energy-generating power plants of living cells.

Tumor necrosis factor inside of infected macrophages induces reactive oxygen species from the mitochondria. These are the chemicals responsible for cell damage from oxidative stress.

Early on, reactive oxygen species can be beneficial. Initially their presence encourages the macrophages to destroy pathogens. As they accumulate, however, they promote self-harm.

Suddenly the macrophage is programmed to self-destruct. The reactive oxygen species carry out the death sentence by modulating a pathway for a substance called cyclophilin D, which sets the stage for the demolition of mitochondria.

Reactive oxygen species also play a role in acid sphingomyelinase-mediated ceramide production. This waxy substance occurs in cell membranes. One of its many roles is regulating signals for cell death.

The researchers were able to convert the high tumor necrosis factor state to become resistant to tuberculosis. They did so by genetically blockading both cyclophilin D and acid sphingomyelinase in previously susceptible zebrafish.

Similarly, they discovered that the drug combination of alisporivir, a cyclophilin D-inhibiting drug, and desipramine, an antidepressant that inactivates acid sphingomyelinase, also reverses susceptibility to TB in

zebrafish prone to tumor necrosis factor excess.

Essentially, the experiments suggest that preventing cell death in TB infected macrophages can prolong their capacity to attack TB pathogens.

A longer-living army of macrophages, filled with the microbiocidal reactive oxygen species, will destroy the TB pathogens inside them and make the host highly resistant to tuberculosis.

Because excessive amounts of tumor necrosis factor are implicated in several inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis, sarcoidosis, and Crohn's, the authors noted, "The findings may be useful for understanding diseases in addition to tuberculosis."

Provided by University of Washington