Sabine Ehrt: Searching for mycobacterial stress points

By figuring out how mycobacteria survive within the very cells that try to kill them, Ehrt hopes to find a way to combat chronic tuberculosis infections.

For most bacteria, getting scooped up by macrophages means a rapid death at the hands of a lethal cocktail of nitric oxide, free radicals, and destructive enzymes. *Mycobacterium tuberculosis* avoids this fate first by preventing the fusion of phagosomes with lysosomes in resting macrophages. The bacterium then turns off its replication machinery, allowing it to establish a latent infection.

Sabine Ehrt has been grappling with *M. tuberculosis* survival tactics for a decade. During her post-doctoral years with Lee Riley, first at Weill Cornell Medical School (New York) and then at the University of California, Berkeley, she helped identify some of the mechanisms that mycobacteria use to resist the damage caused by free radicals (1).

After moving back to Cornell to start her own laboratory, Ehrt focused on events that occur before the bacteria entrench themselves by focusing on the cellular receptors that detect infection and the signals turned on in response (2, 3). Along with other Cornell microbiologists, she also explored the environment within infected phagosomes by probing the transcriptomes of host cells and pathogens. This effort revealed some of the genes and pathways that are switched on in infected macrophages and provided clues about how mycobacteria modify the cells' transcriptional signature (4, 5).

"The trick to get a good answer with (transposon mutagenesis) is to ask the right question and use the right genetic screen."

Ehrt's team has used various genetic tricks to identify bacterial mutants that cannot combat the macrophages' toxicity (6). Using a mutagenesis strategy, they recently identified a bacterial membrane protein that allows the pathogen to resist acidification, which triggers the activity of bacteria-killing enzymes (7). As bacteria lacking this acid-resisting protein are killed easily in vivo, drugs that target the protein might help fight latent tuberculosis—a disease that affects a third of the world's population.

EAGER TO APPLY

You started your career working on the genetics of soil bacteria. How did you get interested in that subject?

Like most people who go into research, I had always been curious about nature—like why fruits and vegetables have their colors, for example. I remember trying to isolate carotenes from veggies in a little laboratory that I'd set up in our bathroom when I was in high school. I got interested in genetics after that and so for my Masters' thesis, I worked on the tet promoter and its recognition by RNA polymerase. But for my PhD thesis, I wanted to study something that was a little more applicable to real life. So I began a project that combined genetics with environmental utility; I tried to identify genes that allow a type of soil bacteria to eat phenol and other toxic substances and degrade them.

Sounds very eco-friendly. What made you switch to tuberculosis research?

After I finished my PhD, I realized that I liked the applied aspect of science more than anything. Now I wanted to do something that was medically relevant. It was 1994 and tuberculosis had reentered our awareness in a big way. I read that New York City had had an explosion in tuberculosis cases, which motivated me to come here to work on this problem. My application for post-doctoral work was enthusiastically accepted by Lee Riley, who was then at Cornell. I also got a great five-year fellowship from the German Cancer Research Center that would allow me to stay in the US for two years and then go back to Germany to finish up and become a professor there. But I ended up staying in the US for the whole time.

Sabine Ehrt

ROOTING OUT RESISTANCE

What was your post-doctoral project?

Carl Nathan, who collaborated with Lee, had discovered the importance of iNOS—the enzyme that produces nitric oxide that helps kill the pathogen. I tried to find antioxidant genes in mycobacteria. Lee's laboratory had already constructed a mycobacterial expression library in *Escherichia coli*. The idea was to look for recombinant *E.coli* that had become resistant to nitric oxide. A previous post-doc had cloned one of these resistance genes, called *noxR1*, and I analyzed its function and helped others in the group clone similar genes.

After these resistance mechanisms and the genes that drive them had been identified, was there an effort to target them for therapy?

No. The problem was that we'd uncovered these mechanisms by expressing mycobacterial genes in other organisms; so it wasn't in the right physiological context. When the *nox* genes were deleted in mycobacteria, the mutants didn't have a dramatic phenotype, suggesting that there were other compensatory mechanisms.

How did you get around this problem?

After I set up my laboratory, we began to use transposon mutagenesis to hunt for genes in the right genetic context. This

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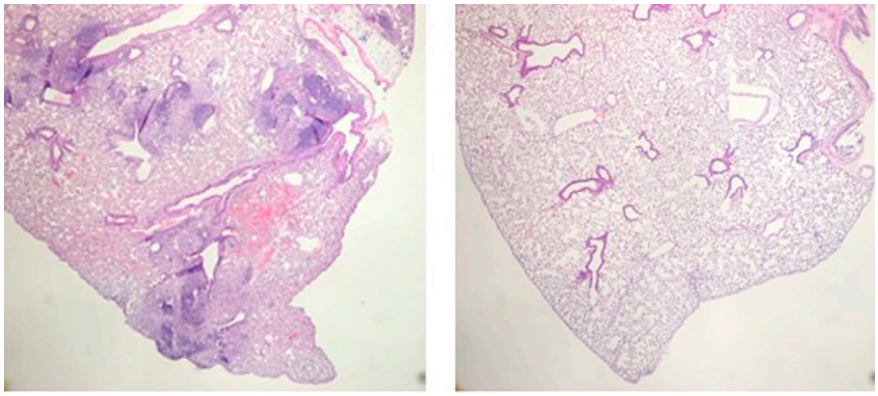

Wild-type *M. tuberculosis* causes more pathology in mouse lungs (left) than the acid-susceptible mutant (right).

way, we could stay with mycobacteria and not transfer genes from these pathogens into *E.coli* or *Salmonella*.

The trick to get a good answer with this approach is to ask the right question and use the right genetic screen. Soon after I came to Cornell, I collaborated with Dirk Schnappinger and Gary Schoolnik to examine the bacterial transcriptome while it resides within the macrophage to understand how mycobacteria respond to environmental stress. One stress is due to low pH, as the phagosome acidifies in response to the main macrophage activator IFNγ. *M. tuberculosis* would have to have some way of defending itself against the acidification process to survive. So we used a transposon library where one gene in each mycobacterium is interrupted by a transposon, and screened this library for mutants that couldn't survive acidification. We came up with 21 mutants; one of the mutations was in a gene that encodes a membrane protein that stabilizes pH within the bacteria even when environmental pH is low.

How does this protein do that?

That remains to be seen. Because the protein has serine protease activity, it might degrade unfolded proteins that accumulate in response to acid stress. Or it might modify the bacteria's cell envelope to defend against acidification.

How does the lack of this protein affect bacterial growth?

Knocking out this protein impairs in vitro growth when subjected to acid stress but not under normal conditions. But in mice infected with these mutants, both bacterial replication and persistence is impaired. This is important because tuberculosis is a chronic, persistent infection in a majority of people. This protein might therefore be a good target for drugs to prevent latent infection.

THE POWER OF COLLABORATION

Aside from resisting stress, do the mycobacteria also influence or modify the reactions of the macrophage?

Our collaborators at a biotech company helped us address this question by examining the transcriptomes of infected macrophages while we were studying that of the mycobacteria. Macrophages were dramatically reprogrammed by mycobacterial infection, and molecules such as iNOS and other oxidative enzymes that were thought to be just defensive turned out to also have signaling functions.

Based on these data, we started to look at Toll-like receptor (TLR) signals in more depth. Our work so far suggests that the adaptor MyD88 controls more than just TLR signals because mice that lack this molecule have much more severe disease than TLR-deficient mice.

Might there be more secrets hidden in that transposon library? Have you come up with other genes that guide other resistance mechanisms?

A post-doc from Carl Nathan's laboratory, Heran Darwin, who actually made the library, identified several proteasome-associated genes and pathways that help the bacteria resist the effects of nitric oxide, which suggested that the bacterial proteasome might contribute to pathogenicity.

Does that mean that the bacteria's proteasome is a valid drug target?

Yes. We've demonstrated this by using conditional gene silencing to turn off the operon that encodes the proteasome in mice after they've developed chronic tuberculosis. Although the operon isn't essential for bacterial growth in vitro, the bacteria need it to survive during the infection's chronic phase. The conditional knock-down of the operon in the mice allowed them to get rid of persistent infection. So Carl's team is now looking for mycobacterial proteasome inhibitors to develop into drugs. They're also looking for chemical inhibitors of the acid resistance protein that I mentioned earlier.

Sounds like a productive collaboration.

I'm a big believer in the power of collaboration. I'm actually part of a huge consortium funded by the Gates Foundation and our goal is to identify drugs to treat latent infection. While groups like mine try to validate drug targets in vitro, in mice or in nonhuman primates, the chemists in the consortium assess the molecules for their druggability—whether these compounds can be turned into drugs. I've learned that working in isolation is not beneficial. Collaborating not only has made science move faster, but also made it so much more fun.

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