QnAs with Ralph R. Isberg

The moment a bacterium invades a human cell, the immune system triggers a series of events meant to destroy the pathogen and display the remnants like victory flags on the cell's surface. National Academy of Sciences member Ralph R. Isberg, a professor of molecular biology and microbiology at Tufts University in Boston, MA, revealed early in his career how some bacterial pathogens evade this process, gaining entry to host cells and continuing to multiply despite the harsh, intracellular environment. Isberg spoke with PNAS about his work on *Legionella pneumophila*, the bacterium that causes Legionnaires' disease. Isberg describes his recent finding that *L. pneumophila* can grow inside human immune cells even when it lacks nearly one-fifth of its genome.

PNAS: *Legionella pneumophila* is a so-called opportunistic pathogen, suggesting that it infects human cells by accident when water aerosols from air-conditioning units and faulty plumbing systems make their way into the human lung. How do such accidents occur?

Isberg: The pathogens we normally think of are those that spread from human to human, like the cause of strep throat. However, in the case of *L. pneumophila*, you're not getting it from an animal, you're not getting it from another human, you're getting it from bad water. Although it sounds almost medieval, like there's some kind of bad humor there, it's true because the bad water is harboring amoebae, and the bug is living in the amoebae. Thus, you have an organism that's figured out how to grow very well in amoebae, and fortuitously some of the factors that were selected for growth in amoebae also allow this organism to grow in people whose immune systems are compromised in some way.

PNAS: Most bacteria cannot survive inside human cells; in fact, the immune system relies on this limitation to degrade and clear pathogens from our bodies. What factors allow *L. pneumophila* to survive within cells?

Isberg: Some of the factors we know, some of them we've stumbled upon, and some of this is speculation. However, the first thing is obvious: The bug grows in amoebae, and there are cells—macrophages—that behave like amoebae in humans. Macrophages are professional eaters and so are amoebae. Macrophages live by ingesting and digesting particles of some kind, then displaying the pieces of the digested particle on the cell

Ralph R. Isberg.

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surface to stimulate the immune response. Amoebae grow by eating microorganisms, usually bacteria. So *L. pneumophila* can clearly grow in those cells.

PNAS: Researchers now know that parts of the human genome once thought of as "junk" are quite important. However, in your 2011 Inaugural Article in PNAS, you found that *L. pneumophila* could grow inside human cells, despite lacking 18.5% of its genome. How did you make this finding?

Isberg: We stumbled on this while trying to do something else, actually. The bacterium is able to grow inside cells and it's also able to grow on Petri dishes. If you can't isolate mutations in a particular gene when you're trying to grow the bug on a Petri dish, that means the gene is probably essential. Therefore, we just scanned through the genome and said, "Can we isolate a mutation in this gene?" We found 588 genes where we could not isolate a mutation, so we knew those are essential. We looked at the genome to see where these genes were, and this is where we got a surprise: There were large blocks of the genome where we could absolutely isolate mutations in every single gene. For example, there was a region with over 200 genes in a row, lined up on the chromosome, and every single one of them had mutations in them, which meant that we could isolate mutations in every single one of these genes

and the bacteria would still be able to grow on a Petri dish.

PNAS: What made this finding so surprising?

Isberg: Most people in the field of microbial pathogenesis are married to a model that says the genes associated with causing disease are separate on the chromosome from those necessary for growth on Petri dishes. I could draw the different types of genes as colors and say, "Can you see these yellow islands in a sea of blue?" And of course you would say, "Yeah, yeah, looks beautiful, Ralph. Let's delete the yellow parts and the bug won't be able to grow in macrophages." So we thought if we just kept deleting and deleting and deleting, we'd get a microorganism that couldn't grow in macrophages but would still grow in a Petri dish. Therefore, we made one deletion, two deletions, and so forth until we built a mini-*Legionella* that had five deletions and was missing a large number of genes. What did we find? It grew in macrophages.

PNAS: So why the extra DNA?

Isberg: Well, we found that this mini-*Legionella* grows extremely poorly in all of the amoebae strains we use in the laboratory. However, if you go back and just look at a deletion by itself, we found that it was unable to grow in one amoebae but it was able to grow in other types of amoebae. That extra DNA is not there to allow growth in macrophages; *Legionella* expanded its genome so it can grow on as many amoebae species as possible. Thus, the answer is that these are not pathogenicity islands, they're what we could call "host-range expansion" islands. The islands make it so that the organism doesn't have to specialize in a particular host, like a human or an amoebae species, but it's able to become what we call a "generalist," which means the organism can be extremely flexible and grow in a lot of different species.

It's interesting because you have this organism that's minding its own business growing in amoebae, and human beings have interrupted everything by turning their air-conditioning ducts into pond water and having sludgy plumbing systems, and this stuff is aerosolizing. We already know that compared with amoebae, macrophages aren't a very tough environment for these guys, they don't even need most of their genes to grow in a macrophage. So the bug is all set, ready to go.

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