

## MICROBIOLOGY

# TB Bacteria May Reign Over Cells Intended to Bridle Them

Even before scientists identified the agent that causes tuberculosis (TB), they could tell when it had invaded a person's body from the presence of hallmark lesions called granulomas. These nodules wall off the trouble-making microbe *Mycobacterium tuberculosis* and contain immune cells called macrophages. Conventional wisdom holds that granulomas protect the host, but some work has hinted that they promote bacterial multiplication early in infection. Now, Lalita Ramakrishnan of the University of Washington, Seattle, and graduate student Muse Davis report in the 9 January issue of *Cell* that mycobacteria harness granuloma formation, recruiting new macrophages to the structures and then manipulating the cells into offering the bacteria new quarters in which to reproduce.

Although scientists already realized that granulomas help the TB microbe in the sense that they enable it to hide away and break out decades later, “the belief was that the granuloma is part of a good immune response; it benefits the host,” says microbiologist William Bishai of the Johns Hopkins School of Medicine in Baltimore, Maryland. Ramakrishnan “turned the central dogma on its head.” Andrea Cooper, an infectious disease immunologist at the Trudeau Institute in Saranac Lake, New York, says the new study “redirects our thinking” to considering how the TB bacterium interacts with macrophages early in infection. The work, she and others say, might open new therapeutic avenues and yield clues about why 90% to 95% of infected humans remain symptom-free for life.

The Seattle pair studied *M. marinum*, a relative of *M. tuberculosis*, in zebrafish embryos, an experimental organism whose power derives in part from its transparency. This attribute allows researchers to literally see how bacteria and immune cells behave, even immediately after exposure, when few microbes are present. The study's medical relevance isn't fully known, as *M. marinum* is not *M. tuberculosis*, and zebrafish are not humans, but “the work raises the question of

whether we've been thinking the wrong way” about granulomas, says microbiologist Eric Rubin of Harvard School of Public Health in Boston.

Davis and Ramakrishnan injected fluorescent *M. marinum* into a cavity above the brain, a site that lacks macrophages in the absence of bacteria, and then recorded video of what happened. Macrophages rapidly arrived, ingested bacteria, and traveled into brain tissue. Within 4 days, collections of infected macrophages—early granulomas—appeared. To distinguish whether these structures form when infected macrophages recruit fresh, uninfected ones or when collections of infected macrophages amass, the researchers waited for an initial group of macrophages to engulf

show up consume chunks of the infected cells, thereby ingesting the bacteria inside. “The key point is that one dying cell is infecting a lot of other cells,” says Cooper.

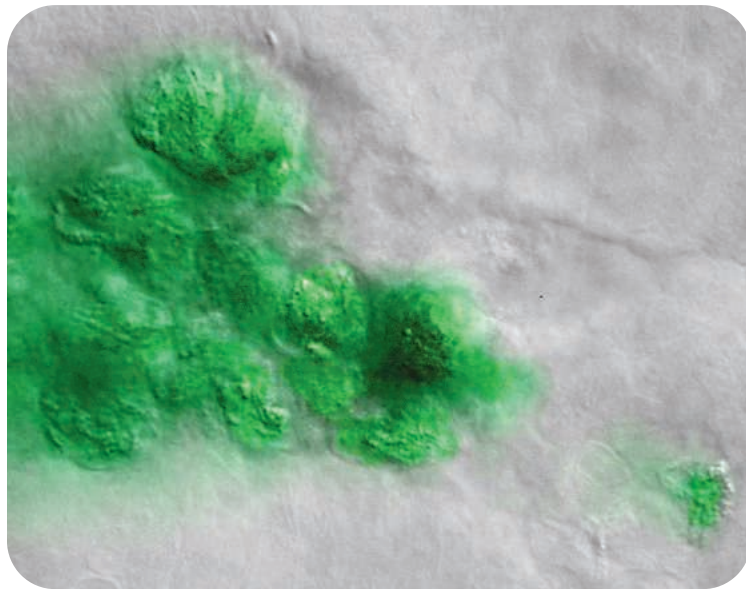
As uninfected macrophages home to the granulomas, they display characteristics of cells that are swimming toward chemical attractants, the researchers observed. The team also discovered that *M. marinum* without a particular region of its genome—RD1—fails to spur key events associated with granuloma formation. RD1 was known to enhance virulence, but no one knew exactly how. The new results suggest that this stretch of mycobacterial DNA somehow triggers granulomas to release a chemical signal that attracts uninfected macrophages. “Here's a clear indication of what RD1 does early in an intact animal,” says Cooper.

Additional experiments revealed that macrophages can escape from early granulomas and seed new ones. “Some macrophages serve as taxicabs to bring [bacteria] to locations where new granulomas can form,” says Bishai. Further analysis revealed that this process accounts for most, if not all, granulomas that appear elsewhere. This observation contradicts a popular theory, which posits that the bloodstream carries free mycobacteria around the body. Whether the strategy operates in other tuberculosis models is unknown, but preliminary results from monkeys are consistent with the dissemination scheme outlined in the zebra-

fish work, says JoAnne Flynn, an immunologist at the University of Pittsburgh School of Medicine in Pennsylvania.

Studying the RD1 system could generate insights about why most people infected with *M. tuberculosis* don't get sick, suggests Ramakrishnan. Individuals who resist disease may, for example, not respond to RD1's influence. The work might also point toward clinical interventions that quash infection before it takes hold, she says. “If you could intercept the pathway that RD1 uses to lure macrophages, you would have a whole new approach to treating TB.”

—EVELYN STRAUSS



**Hitching a ride.** Infected macrophages (green) can leave established granulomas (left) and seed new ones at other sites in the body.

microbes and then injected a blue compound that marks macrophages into the bloodstream. Because this substance does not cross the blood-brain barrier, any dye found in the brain must have been carried by cells that came from elsewhere. Uninfected animals' brains remained largely colorless, whereas brains of infected animals held numerous blue, infected macrophages within multiple granulomas, indicating that those cells had been recruited by the first responders.

When infected cells in early granulomas die, they retain their contents, the researchers found. The uninfected macrophages that then