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## Unexpected Power from Killer T Cells

Howard Hughes Medical Institute researchers have found that killer T cells -- the sentinels of the immune system -- possess a hidden strength that may be used to improve vaccine design for tough-to-beat bugs, such as *Staphylococcus aureus*.

The new experiments show that killer T cells can attack bacteria that attach to the outside of cells. Prior to this work, immunologists thought that killer T cells only attacked cells that had been invaded by bacteria and other pathogens, said Howard Hughes Medical Institute investigator Ralph Isberg, who is at Tufts University.

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— **Ralph R. Isberg**

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"Killer T cell responses have long been associated with pathogens that grow within host cells," says Isberg. "But we were surprised when we found that killer T cells were really important for protection against this extracellular bacterium."

Isberg and colleagues found that these T cells are vital for clearing mice of infection with *Yersinia pseudotuberculosis*, a gut-invading bacterium that microbiologists often use to study the immune system. *Y. pseudotuberculosis* attaches itself to the outside of cells in the gut.

Molly Bergman, a postdoctoral fellow in Isberg's laboratory, conducted most of the work on the study, which was published September 4, 2009, in *PLoS Pathogens*.

Bergman began the research by inoculating mice with a crippled strain of *Y. pseudotuberculosis*. The impaired pathogens in that inoculation acted as a vaccine, priming the animals' immune systems to fight off infection when they were later injected with a fully active strain of the bacteria.

When Bergman examined the inoculated mice, she found high levels of anti-Yersinia antibodies – an expected finding. Antibodies and the cells that make them form one arm of the mammalian immune system, acting as a long-term memory that recognizes and tags pathogens that have previously infected the organism. However, Bergman also noticed an increase in the number of activated killer T cells in the inoculated animals. Killer T cells play a key role in the second arm of the immune system, known as cell-mediated immunity, by homing in on infected host cells that display fragments of a pathogen on their surface.

Next, Bergman injected crippled *Y. pseudotuberculosis* into mice lacking killer T cells. Even though these bacteria were not full-strength, they made the mice sick -- spreading to the lymph nodes, spleen, and liver. The bacteria colonized the spleen or liver of all the modified mice, while only a few of the normal mice were infected. Eventually, all of the mice lacking killer T cells died, while all of the normal mice survived.

“It was a head-scratcher,” says Isberg. “You have these killer T cells that normally kill infected host cells, but now it looked like these same cells also protected against an extracellular pathogen. Nothing in the immunology literature could explain this.”

Bergman delved further to examine the role of a critical protein, called perforin, which is made by killer T cells. When a killer T cell encounters an infected host cell, it squirts perforin at the infected cell. Perforin penetrates the cellular membrane and opens a channel for destructive enzymes that eventually kill the cell. Like the killer-T -cell-deficient mice, mice lacking perforin succumbed to *Y. pseudotuberculosis* infection more frequently than normal mice. More perforin-deficient mice than normal mice died from infection. So perforin itself, not just the killer T cells that made it, was crucial in protecting against infection.

Bergman then conducted a series of tests in cell culture to study how the killer T cells were inhibiting infection. In these experiments, she found that after a killer T cell attacks a cell that is carrying *Y. pseudotuberculosis*, other immune system cells called macrophages engulf the dead cell and all the bacteria that live on its outer membrane. It’s as if the killer T cell indirectly tags the bacteria for destruction, Isberg explains.

“When this happens, the bacterium is no longer locked on the outside of a host cell, where it can replicate and continue to harm the host,” he says. “Instead, the host cell and all of those bacteria -- the whole gemish -- is now internalized in a macrophage, which digests all of it. What’s being recognized by the macrophage is not the bug itself but the dead host cell.”

Isberg calls this mechanism the “three cell model” of immune protection against extracellular pathogens. The first cell is the host cell that carries the bacteria. The second cell is the killer T cell, which kills the host cell. The third cell is the macrophage, which engulfs and digests the whole complex.

Because many tough-to-treat bacteria, such as *S. aureus*, are extracellular, Isberg sees important implications for vaccine development. “Now it looks like you might be able to stimulate the immune response in different ways, so you get a synergistic effect between cellular immunity and humoral immunity [antibodies],” he says. “That might be helpful for developing vaccines for extracellular pathogens in a way people hadn’t considered before.”