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Handicapping Tuberculosis May be the Way to a Better Vaccine

Howard Hughes Medical Institute investigator William R. Jacobs and colleagues have produced a genetically altered strain of tuberculosis (TB) that elicits a stronger immune response than the current vaccine, bacillus Calmette-Guérin (BCG). The new vaccine improves survival of infected animals and may help put scientists on track to replace BCG, which has been used for nearly a century although it is largely ineffective.

Despite widespread vaccination programs, the World Health Organization estimates that approximately 2 billion people worldwide are infected with TB, with over 95 percent of infections occurring in developing countries. Most TB is latent, but can become active when the immune system is weak, such as during HIV infection, and more than 1.6 million die each year from this disease. In addition, resistance to current treatments is becoming increasingly common, making an effective vaccine all the more crucial.

"We're very excited because this is the first vaccine strain we've ever seen that is significantly better than BCG," said Jacobs.

"The pathologist we sent these lung sections to was amazed. He said he'd never seen anything like it."

- William R. Jacobs Jr.

Jacobs worked in collaboration with Steven Porcelli at the Albert Einstein College of Medicine. The team will publish its findings in the August, 2007, issue of the *Journal of Clinical Investigation*.

The immune system has a very difficult time detecting and combating *Mycobacterium tuberculosis*, the bacteria responsible for tuberculosis. By creating a strain of TB that behaves differently, Jacobs believes he can build a vaccine that prepares the body to recognize TB when infected.

If a cell becomes aware that a pathogen has invaded, it can prevent the infection from spreading to other cells by committing cell suicide—a process known as apoptosis, where the cell shuts down and is shredded into little pieces.

Immune system cells further chop up these cellular bits and present them to white blood cells called T lymphocytes. Each T lymphocyte specifically recognizes a different small piece of protein, known as a peptide. Upon encountering their specific peptide, T lymphocytes multiply and mount an attack against the pathogen.

After the pathogen has been eliminated, most of the T lymphocytes die, but a small population continues to circulate throughout the body. These cells are known as memory T lymphocytes, and will be poised and ready should the pathogen appear again. The most effective vaccines induce the immune system to produce memory T lymphocytes, which are crucial to establishing life-long immunity.

Of course, pathogens have tricks to evade the immune system. *M. tuberculosis* lives in the lungs, in immune cells called macrophages. A weapon in TB's arsenal is an enzyme called superoxide dismutase A, or sodA. This enzyme helps TB cover its tracks, so the macrophage doesn't know it's infected. Jacobs and his team hypothesized that eliminating this enzyme's activity would give macrophages the opportunity to trigger apoptosis, thus prompting a more effective immune response.

So the researchers deleted the gene responsible for shuttling sodA out of the bacterium, effectively disabling sodA activity. When they compared this mutant strain to normal TB, they saw that it did in fact cause increased apoptosis in macrophages grown in culture.

To get a better picture of what was going on, Jacobs and his team used a mouse that had been genetically altered to have all of its T lymphocytes recognize the same peptide. They altered the normal and the apoptosis-inducing strains of TB to produce this peptide.

The researchers transferred thousands of immune cells from the genetically-altered mice into normal mice, which they then infected with the two strains of TB. This procedure gives scientists a magnified view of the immune system's response. Although both strains of TB produce the same amount of peptide, Jacobs expected infection with the apoptosis-inducing strain to elicit a more dramatic immune response.

The researchers observed that cytotoxic T lymphocytes (CTLs), which are needed for eliminating a pathogen and establishing a memory response, proliferated much more in the mice that had been infected with the apoptosis-inducing strain. This means that the immune system was able to detect and respond to infection by the mutant strain better than normal TB.

From here, Jacobs compared his mutant strain with the current vaccine, BCG, to see if it would be more effective. The scientists injected mice with the mutant TB or BCG and then infected them with an aerosolized form of TB two months later.

The results were impressive, said Jacobs. One month after infection, the lungs of mice that had been vaccinated with the mutant strain and then infected with TB looked like they'd never had TB at all. "The pathologist we sent these lung sections to was amazed," said Jacobs. "He said he'd never seen anything like it."

A year after infection with TB, less than 25 percent of the BCG-vaccinated mice had survived. In contrast, over 75 percent of the mice vaccinated with the mutant strain were still alive. Even beyond the course of the experiment, the mice have continued to thrive. "It's a year and a half later now, and those mice are still kicking," Jacobs said proudly. The team had the same successes when they repeated the vaccination experiments in guinea pigs, which are highly susceptible to TB infection, further encouraging the notion that this vaccine may be effective in humans.

Jacobs is optimistic about the research, but he knows there is still much that needs to be done. His team is already working to modify the strain to make it less virulent, putting them one step closer to replacing BCG.