Peroxynitrite radicals can damage the cell's proteins by adding nitrate groups to the amino acid tyrosine. In work that has not yet been published, several groups, including Beckman's and Gurney's, have found such nitrated tyrosines in cell proteins in tissue samples from both ALS patients and mice. The targets include the neurofilaments, protein bundles needed for neuronal structure and function. Indeed, neurobiologists suspect that both peroxynitrite and peroxidation may be involved in damaging neurons. The two ideas are "absolutely not competing," says neurobiologist Sam Sisodia of Johns Hopkins University. "They're both within the realm of possibility."

Despite the recent progress, researchers still have a long way to go to understand what causes ALS. Bredesen notes, for example, that no one has demonstrated directly that membrane lipids are attacked by CuZnSOD peroxidase. And then there is the biggest question of all: whether the information being gleaned from the studies will lead to effective ALS therapies. Bredesen's and Gurney's results suggest, for example, that treatment with antioxidants might delay development of ALS in individuals carrying SOD1 mutations, although a great deal more work will be needed to prove that. As Brown says, "This is a story that is still very much in its infancy. It's still evolving." But, he adds, "That's what's exciting."

–Jean Marx

IMMUNOLOGY

Modified Microbe May Boost TB Vaccine

BOSTON—In medicine's war against bacterial infections, one of its longtime allies has been a member of the enemy: bacille Calmette-Guérin (BCG). The relatively benign mycobacterium has been widely used as a vaccine against its more malicious cousin, the tuberculosis (TB) organism, because the similarity between BCG and TB proteins appears to arm the body's immune system against a subsequent TB attack. But BCG's protective effect varies greatly among different populations and wanes as people age. Now reinforcements may be on the way, in the form of the intercellular messengers known as cytokines, which can rally the immune system.

A group of researchers in Cambridge, Massachusetts, reports engineering BCG to express several mouse cytokines that stimulate immune cells such as macrophages and T cells to begin an all-out immunological assault on the invader. In this week's issue of the *Proceedings of the National Academy of Sciences*, molecular biologist Richard Young of the Whitehead Institute for Biomedical Research and his colleagues say that inoculating mice with the recombinant bacterium greatly strengthened their cells' immune responses to tuberculosis antigens.

The accomplishment raises the prospect of an improved vaccine against the worldwide TB epidemic and is winning accolades from other researchers. "A tremendous achievement," says Barry Bloom, an immunologist at the Albert Einstein College of Medicine in New York who specializes in the study of TB and who collaborated with Young on earlier studies of BCG. Moreover, the research could lead to more effective cancer therapies, because conventional BCG spurs the immune system to attack some bladder tumors, and scientists believe a recombinant form could provide an even greater stimulus. The technique, however, hasn't yet proven itself against TB or cancer, even in animals. Still, Kenneth Stover, a molecular microbiologist at the Seattle firm PathoGenesis, says that "it's potentially exciting. There is definitely room for an improved BCG vaccine, and this may be a much cheaper, safer way to do it than adding cytokines as drugs."

Slow-growing BCG has been used as a live, attenuated TB vaccine for about 50 years outside the United States, where it is not approved because the organism's resemblance to TB produces false positives in TB screening tests. Three years ago, Young's team, including immunologist Peter Murray of the Whitehead Institute and molecular virologist Anna Aldovini of Boston's Children's Hospital, hit on the idea of enhancing its effects on "cell-mediated" immunity—the immune response that calls on cells such as T cells and macrophages to strike back at pathogens. Manipulating BCG to produce its own mammalian cytokines, they realized, might enable it to stimulate a stronger response by these immune system cells.

But the researchers were not sure that the mycobacterium could be made to produce and secrete cytokines, complex proteins that are biologically inactive unless their multiple units are bound and folded in the proper configuration. There was a safety issue as well, says Young. The bacterium might overproduce the cytokines, which could be toxic and actually compromise immune system functioning.

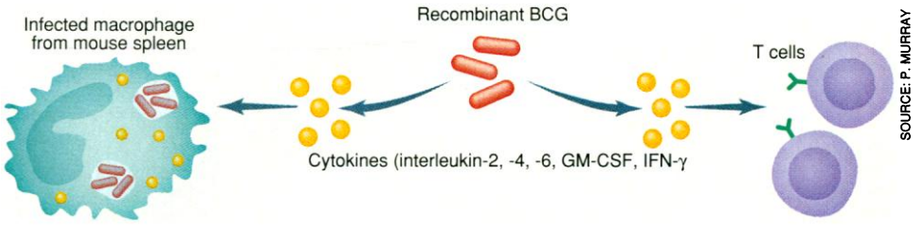
The group designed a two-part experiment to test these questions. They first inserted mouse genes encoding many different cytokines behind bacterial promoters, or "on switches," and signal sequences that ferry proteins through the bacterial cell wall. Progress was slow—mycobacterial strains such as BCG are notoriously difficult to grow—but the genetically engineered bacteria delivered the goods. Says Young, "We were able to secrete in active form a number of the cytokines we tried," including interferon-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-2, -4, and -6.

The researchers then injected these strains into mice. Not only did the mice remain healthy, but their immune systems still functioned properly—and showed a strengthened response to TB antigens. T cells later isolated from their spleens responded to purified tuberculosis proteins by proliferating and producing cytokines at rates up to 10 times higher than normal. The researchers now plan to test whether the cytokine-producing BCG strains are capable of strengthening immunity to the actual tuberculosis bacterium in experimental animals.

The Whitehead team's advance is also encouraging cancer researchers such as Michael O'Donnell, a urologist at Boston's Beth Israel Hospital, who hopes that the recombinant bacterium will prove to be a more powerful immunotherapeutic agent against cancer. In body cavities where BCG can be injected and confined, such as the bladder, the bacterium has been shown to eliminate superficial tumors, possibly by provoking an antibacterial response that also purges tumor cells. Recombinant BCG may be able to augment this process, O'Donnell says.

He is already testing the new BCG strains' effectiveness against tumor cells in vitro, with promising preliminary results. "Suddenly you have an organism that can stimulate a response in itself, and can be directed and focused by the incorporation of ... cytokines," says O'Donnell. "It's an incredible asset."

–Wade Roush



Booster bug. Strains of bacille Calmette-Guérin (BCG), engineered to express mammalian immune system messengers called cytokines, stimulate an enhanced response to tuberculosis proteins.