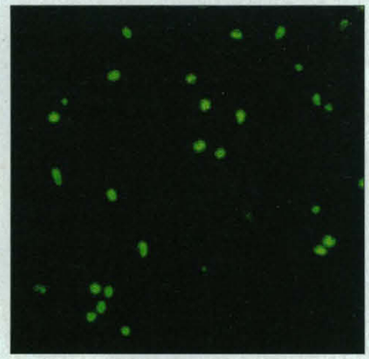
MICROBIOLOGY

Gene May Promise New Route to Potent Vaccines

For protection against an infectious disease, few things can beat a vaccine made of the living organism. But every live vaccine is a balancing act: The pathogen has to be vigorous enough to trigger an immune defense by the host, yet too weak to lead to serious illness. On page 967 of this issue, a team from the University of California, Santa Barbara, comes up with a new answer to the problem. They have found a gene that seems to orchestrate the activity of dozens of other genes needed for a full-blown infection by *Salmonella typhimurium*, a bacterium that causes food poisoning in humans and a typhoidlike disease in mice. When they knocked out the gene, the bacteria became powerless to cause disease but still elicited a fiery immune response in mice—in other words, they had apparently become the ideal vaccine.



Dam spots. A fluorescent antibody signals activity of the Dam protein in *Salmonella* cells.

Because the gene, which produces a protein called DNA adenine methylase (Dam), is shared by many other pathogens, the researchers think that easy-to-produce vaccines for a range of diseases, from meningitis to the plague, may lie within reach. For some of these, no vaccine is currently available. "We believe this may have tremendous impact in the field of infectious diseases," says geneticist and lead author Michael Mahan. Other researchers agree that the work may result in a flurry of new studies, but some are cautious. "It's an important and tantalizing clue," says John La Montagne, deputy director of the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, "but whether or not it can work in humans is the $64,000 question."

Pathogens like *Salmonella* have many so-called "virulence genes," which are switched off when the bacteria are living on a petri dish or a chicken in the refrigerator, but spring into action once they enter the gut of a mammal, where they help the bacterium to penetrate the gut lining, travel throughout the body, and use the host's resources to grow and divide. Mahan's team had already discovered some 250 of these genes in *Salmonella*. Little was known about how they are regulated, although earlier studies had shown that a protein called PhoP controls the expression of some of them.

In their search for other regulators, the team tried many candidates. One of them was Dam, which was known to be involved in DNA repair. In *Escherichia coli* strains that cause urinary tract infections, Dam also controls the formation of pili, hairlike protrusions specialized in latching onto host cells. To see whether the protein might have a wider role, the team created an *S. typhimurium* strain that lacked the *dam* gene and found that it was utterly innocuous to mice, even in huge doses; when administered orally, the bacteria entered the mucosal tissue lining the gut but didn't colonize organs such as the liver and the spleen. Measurements of gene activity showed that the absence of Dam altered the expression of at least 20 virulence genes, and Mahan says further experiments have shown the real number to be at least 40.

Dam apparently acts as a master switch for these genes, because it can glue methyl groups to DNA strands at specific sites. By doing so, says geneticist and co-author David Low, the enzyme decreases the ability of some regulatory proteins to bind to DNA, while increasing that of others; each of these proteins can in turn crank up or slow down the transcription of one or more genes.

The authors say the finding may give scientists several new weapons in the relentless race against bacterial infections. For one, drugs that block Dam could slow down bacterial growth and possibly result in a whole new generation of antibiotics. And when the team immunized 17 mice with Dam-negative *S. typhimurium*, they found that the strain is an effective vaccine. The immunized mice all withstood terrifying doses of the normal bacterium 5 weeks later—up to 10,000 times the amount that killed nonimmunized mice.

To explain how such enfeebled bacteria could provoke such a strong immune response, Mahan theorizes that knocking out *dam* actually makes the bacteria easier for the immune system to detect. Normally, he says, bacteria turn every gene on only as briefly as possible, to prevent the host's immune system from detecting and attacking foreign proteins. But with the Dam switch shut off, some genes may be expressed for a very long time, within easy sight of the host. "The bacteria become like poker players who are forced to show their cards," says Mahan. "They can't win."

Because different *Salmonella* strains probably share quite a few proteins, one Dam-negative strain may even elicit an immune response that also covers others. In as-yet-unpublished work, says Mahan, the team showed that mice immunized with *S. typhimurium* were also immune to a related *Salmonella* strain that infects chickens and eggs. The reverse was also true, and Mahan is testing whether the vaccine covers more of the 2500 *Salmonella* strains currently known. If it does, inoculating cattle and chickens with a vaccine based on the technique may help banish *Salmonella* from the food chain, he says—although it will have to compete with other vaccines in various stages of development.

Because genetic studies have shown that other gut-colonizing bacteria like *Vibrio cholera*, *Haemophilus influenzae*, *Yersinia pestis*, *Shigella*, and *Treponema pallidum* (which cause cholera, respiratory tract infections and meningitis, plague, dysentery, and syphilis, respectively) all have *dam*, perhaps they too can be crippled by knocking out the *dam* gene. "On the heels of this paper, a variety of labs will probably quickly knock out [*dam*] in their individual bugs," predicts microbiologist John Mekalanos of Harvard Medical School in Boston. Mahan, whose lab has recently gone into overdrive, has already started experiments with four of them, and the researchers have just started their own company specializing in vaccines and antimicrobials. Says Mahan: "I have a hard time believing that it's only going to work in *Salmonella*." –MARTIN ENSERINK

BIOMEDICAL PATENTS

Startling Revelations in UC-Genentech Battle

Just before midnight on New Year's Eve, 1978, Peter Seeburg, then a young researcher at Genentech Inc. of South San Francisco, says he secretly removed a bacterial clone from a lab he had recently left at the University of California (UC), San Francisco, and transferred it to his new employer. That clone, Seeburg testified in court last month, helped lead to one of Genentech's early achievements as a

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