Swallowing *Shigella*

Can bacteria that cause food poisoning deliver oral DNA vaccines?

By JOHN TRAVIS

Shigella flexneri, one of many microorganisms that cause food poisoning, is an unpleasant bug. Fond of invading cells that line the intestines of humans, the bacterium causes gastrointestinal illness marked by diarrhea, stomach cramps, and fever that can last for a week or more.

This type of *Shigella* is also one of the most infectious bugs around: An encounter with as few as 10 of these bacteria is usually enough to infect a person.

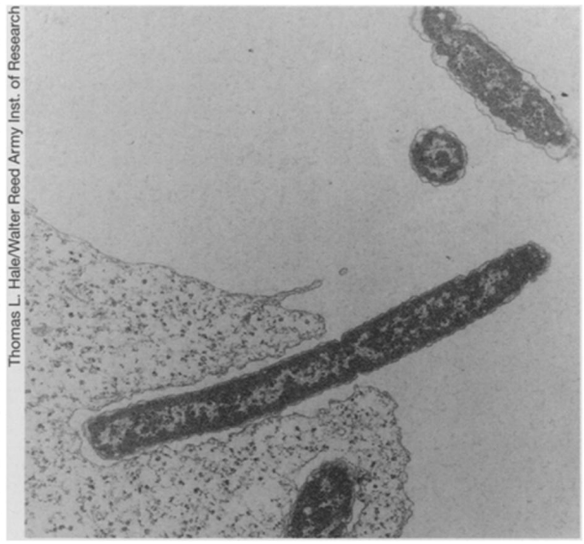
Nevertheless, Jerald C. Sadoff envisions a future in which adults and children, particularly those in developing countries, swallow a pill containing *S. flexneri* to ward off infectious diseases ranging from tuberculosis to AIDS. Sadoff, a bacteriologist now at Merck Research Laboratories in West Point, Pa., isn't crazy. He's merely one of many researchers pushing the envelope of a radical new form of immunization called DNA vaccines.

A number of investigators have recently proclaimed DNA vaccines the third revolution in vaccinology. The first, which celebrates its 200th anniversary this year, occurred when Edward Jenner demonstrated that inoculations with the cowpox virus protect humans from the ravages of smallpox. For decades, immunologists followed Jenner's lead, creating vaccines based either on microorganisms that infect other species or on weakened versions of the ones that cause disease in humans.

The second vaccine revolution took place recently, as the tools of molecular biology enabled researchers to identify and isolate specific antigens—usually proteins or protein fragments—from viruses, bacteria, and other infectious agents. Injections of some of these antigens have generated immune responses to the organisms from which they are derived. The vaccine for hepatitis B, for example, uses a protein found on the surface of that virus.

Over the last few years, in the face of initial skepticism, many research groups have shown that DNA vaccines are a potential alternative to the two traditional forms of vaccines. By simply injecting genes that encode antigens, investigators have stimulated immune responses to the antigens. Cells apparently process the foreign DNA easily, synthesizing the encoded antigens and igniting immunological retaliation against them. The immune responses may be more protective than those obtained by injecting the antigen directly (SN: 1/1/94, p. 6; 6/3/95, p. 343).

Vaccines based on this approach, often called naked DNA vaccines, have taken the first small steps into human trials against AIDS and cancer (SN: 2/17/96, p. 100).



Thomas L. Hale/Walter Reed Army Inst. of Research

In this electron micrograph, a cell engulfs one of three nearby Shigella flexneri. *A fourth bacterium has already been consumed. Vaccine developers add foreign DNA to such bacteria in the hope that, once inside a cell, the bacteria will release the DNA. The cell would then use the genetic material to synthesize a protein that stimulates an immune response within the host organism.*

DNA vaccines have gone "from a really unacceptable form of immunization a couple of years ago to something so promising now," marvels Harriet L. Robinson of the University of Massachusetts Medical Center in Worcester.

The science of DNA vaccines is still in its infancy, however. While most researchers resort to injecting DNA directly with a syringe or using so-called gene guns to shoot DNA-covered gold pellets through the skin, a few investigators, such as Sadoff, have explored the possibility of delivering future DNA vaccines orally.

Oral vaccines are desirable for a number of reasons, including cost and simplicity. Eliminating the expense of needles and of the professionals who provide the injections is a major advantage, especially when it comes to vaccinating millions of people in developing countries. "Oral delivery is ultimately easier," says Sadoff.

Oral vaccines may also provide a different, and more effective, form of protection than many injected vaccines. Injected vaccines place antigens directly in the bloodstream, which becomes the site of most of the protective antibody and cellular immune response.

The bloodstream, however, may not be the best place to generate an immune response against certain microbes. Before they ever reach the bloodstream, many infectious agents must cross an often ignored part of the immune system known as the mucosal barrier.

This thick, antibody-laden mucus protects the cells lining the body's respiratory, gastrointestinal, urinary, and reproductive systems. The mucus can trap invading microorganisms and prevent them from gaining a foothold in the body.

More so than injected vaccines, oral vaccines can strengthen immune responses to a particular microbe within the mucosal surface of the intestine. This reaction, in turn, induces similar responses in additional parts of the mucosal system, particularly the vagina and other regions of the reproductive system.

"There's evidence that the immune responses in these sites are linked in some way that's not well understood," says Christopher Clegg of the Centre for Applied Microbiology Research in Salisbury, England.

An oral vaccine that triggers an anti-HIV immune response within the reproductive mucosal surface is a possible means of preventing the AIDS virus from spreading through sexual contact, notes Clegg.

Turning *S. flexneri* into an oral vaccine is a new tactic for researchers. Bacteria have always been important tools for creators of DNA vaccines, but researchers have normally used the microbes as a simple way to copy DNA, not as a method of delivering it into the body.

To produce enough copies of a gene to use as a vaccine, researchers bend the required DNA into a genetic hula hoop called a plasmid and add these plasmids to bacteria. As the bacteria multiply furiously, the plasmids are copied along with the bacteria's own DNA. Investigators finally kill the bacteria and purify the plasmids for later injection as a vaccine.

Sadoff, then at the Walter Reed Army Institute of Research in Washington, D.C., and his coworkers realized that they could cut a step out of this complex process by having *S. flexneri* both copy and deliver the plasmids.

The investigators had already been working with the idea of using bacteria as a delivery system for antigens. If they inserted the gene for a particular antigen into a harmless strain of bacteria, the reasoning went, the bugs should begin making buckets of the antigen. If a person swallowed such genetically engineered bacteria, he or she might develop a strong immune response to that antigen. The problem, the Walter Reed team found, was that it's not easy to persuade *S. flexneri* to synthesize the immune-stimulating antigens of another organism.

So the Walter Reed group, which included Donata R. Sizemore and Arthur A. Branstrom, decided to abandon that concept and focus on getting the bacteria to infect a host's cells and simply dump any plasmids there.

"Instead of having the bacteria make foreign proteins, have them just be a delivery vehicle for DNA. The bacteria don't mind delivering the DNA, and you get around the problem of forcing an artificial system," says Sadoff.

He and his colleagues discussed this concept, and reported early results based on it, in the Oct. 13, 1995 SCIENCE and in a presentation at an international meeting on DNA vaccines held in Bethesda, Md., last February.

To transform *S. flexneri* into an oral DNA vaccine, researchers must first make it safe for humans. Since the bacteria cause problems only if they reproduce after invading cells, the Walter Reed group deleted a crucial gene that *S. flexneri* needs in order to replicate. They think the bacteria eventually lyse, or fall apart, in the cell without this gene.

"It's so crippled it can't possibly cause any serious problem in humans. The bacteria can't even divide a single time," says Sadoff.

This DNA vaccine strategy has been somewhat difficult to test, notes Sadoff, because *S. flexneri* does not naturally infect species other than humans and a few kinds of monkeys. The researchers have shown, however, that the bacteria can invade mammalian cells in test tubes and dump their plasmid cargo.

Working with live animals, the investigators have demonstrated that *S. flexneri* can infect cells within the eyes of guinea pigs and release their DNA plasmids into those cells. When supplied intranasally to mice, the bacteria actually stimulated an antibody immune response to the target antigen whose DNA they delivered.

The first realistic tests of this oral DNA vaccine strategy are now under way.

"We have fed monkeys one of these vaccines, but we don't have the results yet," says Sadoff.

The Walter Reed team has not been the only one interested in transforming *S. flexneri* into a DNA vaccine delivery vehicle. A research group led by David M. Hone, now at the University of Maryland's Institute of Human Virology in Baltimore, hit upon the same idea independently.

Hone and his colleagues have pursued a different crippling strategy for the bacterium, however. Worried that the gene deleted by Sadoff's group would reduce the infectiousness of *S. flexneri*, Hone's team deleted two different genes necessary for replication.

Both groups may face a stiff task in convincing colleagues that pills of *S. flexneri*, no matter how crippled the bacteria, provide the safest option for oral DNA vaccines. Bacteria are adept at swapping genetic material among themselves, and there is legitimate concern that *S. flexneri* may overcome its inability to reproduce by borrowing the genes it needs from other bacteria.

Some other oral DNA vaccine strategies that avoid such safety questions may soon challenge *S. flexneri*. At the February meeting in Bethesda, for example, Clegg detailed efforts to encapsulate plasmid DNA in microscopic spheres of a biopolymer called PLG.

"It's a very safe and innocuous material that has been used in medicine for a long time," says Clegg. After a week or so in the body, PLG falls apart, a characteristic that has made it a popular choice for biodegradable sutures.

Before the PLG microspheres fall apart, however, they can protect the vulnerable DNA of an oral vaccine from intestinal acids and enzymes. "You're exposing DNA to fairly hostile conditions in the stomach and gut," notes Clegg.

While traveling through those regions, many of the PLG spheres are swallowed by cells in a process called pinocytosis. As the biopolymer slowly degrades, the encapsulated plasmids are released into the cell's fluid interior.

When Clegg and his colleagues introduced PLG-encapsulated DNA into the stomachs of mice recently, they observed an unmistakable antibody response to the antigen encoded by the DNA.

Clegg's initial goal for the PLG strategy is an oral DNA vaccine for measles. While he says that investigators must make the process of encapsulating DNA into PLG more efficient, Clegg notes that the final product, a freeze-dried white powder, should be stable without refrigeration. "I think there's a real chance that this can be easily distributed in the Third World," says the researcher.

Another possible nonbacterial route to an oral DNA vaccine is cochleates, lipid-based structures that derive their name from the Greek word for snail shell. Cochleates form when sheets of natural lipids, suffused with calcium ions, roll up. "They look like little jelly rolls," says Raphael J. Mannino of the University of Medicine and Dentistry of New Jersey in Newark.

Mannino was working at incorporating antigens into cochleates when he and his colleague Susan Gould-Fogerite decided to examine whether cochleates might also deliver DNA effectively.

The two sprayed plasmid-containing cochleates into the noses of mice. The cochleates then released the DNA into cells, apparently by fusing to their outer membranes. The DNA triggered a discernible immune response, says Mannino.

Currently, the investigators are testing cochleates that contain the gene for a protein that makes up the outer envelope of HIV. Mannino contends that the cochleates should offer an extremely safe option for an oral DNA vaccine.

"These are natural substances. We've never seen any toxicity," he says.

Is there any reason that DNA vaccines, oral or otherwise, couldn't fulfill the promise of a third vaccine revolution? One concern is that the foreign plasmids might disrupt the function of a cell's normal genes.

Investigators have closely examined this possibility in cell and animal experiments and in the few human trials now under way, but all evidence indicates that the foreign plasmids do not integrate into a cell's genome, Clegg says.

Nevertheless, he adds, "the experiments are difficult to do in a convincing way. It's a needle-in-the-haystack problem."

But assuming that such safety concerns remain theoretical and that the testing of *S. flexneri* and other oral forms of DNA vaccines bear fruit, people around the world may one day find vaccination much easier to swallow. □

The World Wide Web has an excellent source of general information on DNA vaccines at http://www.genweb.com/Dnavax/dnavax.html.