Triggering the First Line of Defense

Vaccines that activate mucosal immunity, often the body's first chance to ward off infection, have been hard to come by. That situation is beginning to change. And these new vaccines are needle-free

For decades, vaccine researchers have been fighting infectious diseases without much help from one of the body's major defensive weapons. Most successful vaccines to date, such as the childhood measles, mumps, and rubella immunizations, have been made from injected antigens that spark the body to produce blood-circulating, or serum, antibodies against disease-causing organisms. But the part of the immune system which churns out 70% of the body's antibodies has been virtually ignored, because little was known about how it works.

That part is the mucosal immune system. Membranes covered with mucous (a microorganism-trapping gel) line the airways, the reproductive system, and the gastrointestinal (GI) tract, and many pathogens, such as the bacterium that causes cholera and the virus that causes AIDS, first encounter the body there. "The mucosal surface is the first port of entry of many diseases," says George Lowell, a vaccinologist at the Walter Reed Army Institute of Research in Washington, D.C., who is trying to develop mucosal vaccines. "The hope is that [mucosal vaccines] can stop the virus or bacteria before it gets in."

Now, it seems, researchers are figuring out ways to bring this weapon to bear. As immunologists gain knowledge about the mucosal system, vaccines aimed at eliciting mucosal immunity are coming on line. Those furthest along are typically made from live organisms that have been genetically altered, or attenuated, to insure that they are no longer capable of causing disease. One such anti-cholera preparation recently went on the market in Switzerland and is currently under review in several other European countries. A similar live vaccine for typhoid was approved for use in the United States in 1989. Other live mucosal vaccines are being developed to fight everything from the flu to rabies.

And live preparations are not the only strategies being used. Developers are also cloaking mucosal antibody-triggering antigens in protective coatings to improve their delivery rate or packaging them alongside powerful chemicals called adjuvants which boost antibody responses (see table on p. 1524). "This area is exploding," says mucosal vaccine developer Jerry McGhee, who directs the Immunobiology Vaccine Center at the University of Alabama at Birmingham (UAB).

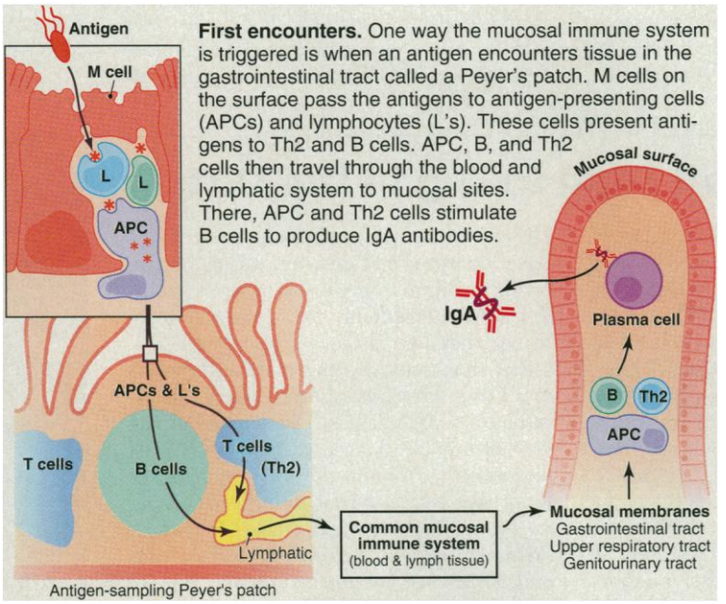

ILLUSTRATION: K. SUTLIFF

Creating an immunological first line of defense is just one reason for the boom. Another is ease of delivery. Researchers are developing many mucosal vaccines to be delivered orally, rather than injected, and oral vaccines represent the Holy Grail to many health experts, says Harvard University microbiologist and vaccine developer John Mekalanos. The United Nations' 1992 Children's Vaccine Initiative calls for oral vaccines for at least 15 diseases, because they are easy to deliver and do away with the need for needles, which can transmit diseases such as AIDS and hepatitis. If mucosal vaccines reach their potential, "it would be the best protection we have," says Marian Neutra, a cell biologist at Harvard who investigates how the mucosal immune system confers protection.

That "if," however, is a substantial one. Mucosal vaccine researchers have been stymied before during the decades they've been working on these preparations, and many of the same obstacles remain. Despite early successes with live attenuated oral vaccines against tuberculosis and polio more than 30 years ago, the expected heyday for mucosal vaccines never followed. One problem was that the chemical attenuation methods then in use altered the genes of pathogens randomly, in many cases changing them enough so that they failed to trigger a vigorous immune response. Other mucosal vaccines, which are usually ingested orally, inhaled, or taken as nose drops, fell victim to destruction by bodily defenses such as enzymes and acids in the stomach, problems not faced by serum vaccines. Finally, in some cases the protection conferred by mucosal vaccines proved fleeting, fading after only a few months or a year.

Such obstacles brought down several mucosal vaccines, including those developed in early attempts to combat cholera and dysentery. And although the base of knowledge about the mucosal immune system has grown, researchers today are still contending with such problems. As a result, most vaccine researchers continue to develop serum vaccines, says Neutra. "It would be nice to have simpler means for administering vaccines," says Mary Lou Clements, who heads the Johns Hopkins Center for Immunization Research. "But mucosal vaccines still need to overcome some hurdles."

Another form of protection

Most researchers' hopes for leaping those barriers lie in their growing understanding of how different immune responses are produced. While serum immune responses involve antibodies of the class known as immunoglobulin G, or IgG, which travel through the blood, immune cells in mucosal surfaces primarily churn out a different type of antibody, IgA, which is released along mucosal surfaces and helps pick invaders off before they can gain entry.

Immunologists have known for some time that the key to producing IgA appears to lie in small clumps of tissue in the GI tract, the nasal and respiratory passages, and other mucosal surfaces that contain various white

blood cells—lymphocytes—responsible for generating specific immunity. In the GI tract, for example, groups of lymphoid cells known as Peyer's patches serve as "quality-control inspectors," sampling bits of food proteins and microorganisms as they pass through the small intestine. Immune cells known as M cells that sit on the surface of the Peyer's patches engulf the small particles and pass them along to a collection of other immune cells lying in the interior of the Peyer's patch: antigen-presenting cells (APCs) and cells called T and B cells. The particles are broken down further by the APCs, which present selected bits on their surface to the B cells, which are the IgA antibody factories (see diagram on p. 1522).

While mucosal immunity typically involves the production of IgA and its release into the mucosal surfaces, antigens picked up by mucosal lymphoid tissues can also trigger the production of blood-circulating IgG antibodies. *Vibrio cholerae*, the cholera bacterium, for example, typically triggers both serum and mucosal antibodies. So in measuring whether a vaccine is effective, what researchers look for is not whether specific IgA levels have risen, but whether a vaccine offers protection against a later challenge by the pathogen.

Going live

To trigger these responses, researchers have begun to design antigen carriers that are specifically targeted to Peyer's patches and other lymphoid tissues that sample antigens. For example, *V. cholerae* is in many ways an ideal carrier, as it naturally colonizes the GI tract and is readily picked up by M cells. The bug's own defenses also make it adept at avoiding destruction by enzymes or stomach acids. Of course, the organism does cause disease, so researchers who want to use it as a protectant need to short-circuit its virulence.

They do so by removing some toxic genes—and it can take a while to figure out how best to do that. In the case of the live attenuated cholera vaccine unveiled in Europe, for instance, University of Maryland (UM) microbiologist James Kaper began work in 1981 to remove the genes from the organism that code for diarrhea-causing toxins, leaving untouched others, such as surface proteins that help trigger an immune response. The advantage of this approach, says UM vaccinologist Myron Levine, who led the team that developed the vaccine, is that "there is much less likelihood that an organism will revert back to a virulent strain by undergoing a random mutation."

It wasn't until the mid-1980s that the UM group was able to produce a strain that was harmless, yet still able to trigger a protective immune response. Now, however, the strategy seems to be working. In trials involving nearly 200 healthy American volunteers, Levine's group showed that just one dose of vaccine provided 100% protection against the moderate and severe diarrhea that is the hallmark of the disease. Those studies were not designed to measure whether the vaccine provided lasting protection; that question, says Levine, should be answered by long-term field trials now underway in 67,000 people in Indonesia.

Researchers are also using live attenuated vaccines to try to trigger mucosal immunity to other afflictions. Several groups, including Levine's group at the University of Maryland and Brian Murphy at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, are attempting to attenuate pathogens to fight typhoid fever and influenza, as well as shigellosis, a major diarrheal disease in developing countries.

Organisms as shuttles

Not every pathogen, of course, conveniently heads straight for M cells when it enters the body. So researchers have begun to introduce DNA snippets from such organisms into the genome of another that is better suited to trigger a mucosal response. The ability of organisms such as *V. cholerae* and *Salmonella typhi* to preferentially bind to M cells makes them ideal carriers. Several groups, including those headed by Levine and by McGhee, are experimenting with *S. typhi* to express proteins from the bugs that cause malaria, diphtheria, whooping cough, and tetanus. They have had preliminary success in getting *S. typhi* to express the new proteins, and animal studies have shown that vaccines made with a similar bug that infects animals can confer protection against challenges.

Another carrier generating interest is Bacille Calmette-Guérin, or BCG, a bacterium originally used as an oral vaccine against tuberculosis. (An injected anti-TB vaccine is currently most used in this country, as the oral vaccine is sometimes difficult to administer to young children.) At MedImmune, a biotechnology firm in Gaithersburg, Maryland, researchers are using recombinant BCG as a delivery vehicle for proteins to fight Lyme disease, urinary tract infections, and tuberculosis. Earlier this year, MedImmune's Solomon Langermann showed that when he delivered recombinant BCG-expressing antigens from *Borrelia burgdorferi*—the organism responsible for Lyme disease—internasally to mice, all of the animals received protection against subsequent challenges by the organism.

These results are particularly striking, Langermann says, because the vaccine triggers not just a mucosal IgA response, but also serum IgG antibodies. Such blood-circulating antibodies are likely to be essential to the success of a Lyme disease vaccine in humans, says Langermann, because the organism makes its way directly into the blood through tick bites. Another plus is that the protection offered by the vaccine seems to last. Langermann's ongoing studies have shown no drop in antibody levels 18 months after administration. That may be due in part to the influence of BCG, Langermann hypothesizes: The BCG vaccine against tuberculosis provides lifetime protection. "But the internasal route may actually be responsible for inducing long-lasting antibody-forming cells in the circulation," he adds. In any case, says Langermann, "I'm hopeful that it will be a good vector against a variety of mucosal pathogens."

One problem with the use of live organisms to deliver antigens from other pathogens is that the patient may develop antibodies against the delivery vehicle. And those antibodies will cause a reaction against that organism if the body meets it again. As a result, most vehicles can be used only once. Such "vector immunity" is a problem for researchers working with *V. cholerae*, says Levine, as there are only two strains, or serogroups, of the organism. This means such organisms will have limited use as shuttles, because a vaccine for one disease could spoil the field for later vaccines that also use *V. cholerae*. Other organisms, like *S. typhi*, contain more than 2000 such serogroups, giving researchers plenty of live delivery vehicles to choose from.

LIZ GILBERT/SYGMA

Vaccines needed. About 20,000 people perished in Rwanda's cholera epidemic this summer. A cholera vaccine that triggered mucosal immunity would have been effective prevention.

Molecular shields

Another way researchers are attempting to shuttle antigens past host defenses such as stomach acids is to encapsulate them in tiny microspheres. The most widely used microspheres are made from a biodegradable polymer called poly (DL-lactide-co-glycolide), or PLG. In the mid-1980s, John Eldridge, then

at UAB and now with Lederle–Praxis Biologicals in West Henrietta, New York, first showed that PLG microspheres could shroud antigens and deliver them to immune cells in the respiratory and GI tracts and generate an immune response. Now researchers are using PLG microspheres to deliver antigens against everything from influenza to diarrhea-causing *Escherichia coli*.

At OraVax in Cambridge, Massachusetts, for example, vaccinologist Tom Monath is experimenting with PLG microspheres to deliver antigens against enterotoxigenic *E. coli*, or ETEC, a principle cause of diarrhea in developing countries. For their anti-ETEC antigen, Monath and his colleagues purified the tiny hairlike cilia that house a "colonization factor" used by ETEC to bind to epithelial cells in the gut. When Monath's collaborators at the Walter Reed Army Institute of Research and UM gave the oral vaccine to healthy volunteers in 1992, they found that two of 10 volunteers were immunized against ETEC. While far from perfect, the fact that encapsulating antigens was effective at all is "quite encouraging," says Monath, who is now preparing for a new round of trials designed to boost the dose of the vaccine in hopes of increasing the percentage of volunteers protected.

In addition to PLG, researchers are experimenting with making microspheres out of other materials, such as liposomes, which are made from the same materials that compose cellular membranes (*Science*, 15 July, p. 316). Many researchers see antigen encapsulation as having a particularly bright future, because the vehicles are being made from common biological materials and typically don't trigger a vector immune response. "I think they are the way of the future, quite honestly," says Mekalanos, who is developing his own live attenuated vaccine against cholera.

Yet, like other delivery systems, antigen capsules have drawbacks. "The missing piece of the puzzle is efficiency," says Mekalanos, explaining that only a few percent of the capsules are taken up by lymphoid cells such as M cells. That's a problem for microsphere vaccines, as in general the less antigen, the less antibody produced. Live vaccines usually sidestep this problem, because they reproduce naturally, supplying ample amounts of antigen.

To boost the uptake of microspheres, researchers control the size of the capsules to between 1 and 10 microns, which for as-yet-unknown reasons are preferentially taken up by M cells. Some groups are also working to target the microspheres more directly. At UAB, for example, Suzanne Michalek is attempting to coat the outer surface of PLG microspheres with the receptor-binding portions of the cholera toxin, which has a special affinity for binding to M cells.

In addition to boosting uptake, researchers are attempting to make the antigens they deliver trigger a stronger immune response by adding chemicals known as adjuvants that heighten the immune response to antigens they accompany. UAB's Ray Jackson reported last year that when mice were given cholera toxin along with antigens from the organism that causes tetanus, all were subsequently protected from a tetanus challenge; without cholera toxin the mice received no protection.

SOME MUCOSAL VACCINES UNDER DEVELOPMENT

Disease	Pathogen	Vaccine Vector
Cholera	*Vibrio cholerae*	Live attenuated and killed bacteria
Diarrhea	Enterotoxigenic *Escherichia coli*	Microspheres, live attenuated bacteria
	Rotavirus	Microspheres, live attenuated virus
	Clostridium difficile	Live recomb. *Shigella*
	Shigella species	Microspheres, live attenuated bacteria
Typhoid fever	*Salmonella typhi*	Live attenuated bacteria
Upper respiratory tract infection	Parainfluenza virus	Microspheres, live attenuated virus
Flu	Influenza virus	Microspheres, live attenuated virus
Pneumonia	Respiratory syncytial virus	Microspheres, live attenuated virus
	Streptococcus pneumoniae	Live recomb. *Salmonella*
Lyme disease	*Borrelia burgdorferi*	Live recomb. BCG
AIDS	HIV	Live recomb. polio virus and *Salmonella*; microspheres, adjuvants
Tooth decay	*Streptococcus mutans*	Microspheres, adjuvants
Meningitis	*Plasmodium falciparum*	Live recomb. *S. typhi*
Sepsis, skin infections	*Staphylococcus aureus*	Encapsulated antigen
Rabies	Rabies virus	Live recomb. adenovirus
Mononucleosis; cancer in AIDS patients	Epstein-Barr virus	Live recomb. adenovirus

Making protection last

Although progress has been made in many of these areas, one hurdle that's proven particularly stubborn for most mucosal vaccines, says Clements, is the need to maintain permanent protection. This clearly isn't impossible in principle. The examples of the oral tuberculosis and polio vaccines show that the mucosal immune system is capable of generating the "memory cells" that remain ready to trigger an immune response if the same invader is encountered later on.

But in some cases, the mucosal immune system doesn't seem to provide this effective memory-based resistance, as certain pathogens, such as respiratory syncytial virus and rotavirus, can reinfect children again and again. "It suggests that mucosal immunity doesn't provide solid protection" against all infections, says Clements. And that may mean researchers will have better luck generating lasting protection against some diseases than against others. Perhaps vaccines that trigger more than one type of immune response, such as recombinant BCG, will help researchers achieve long-lasting protection. But to become commercially viable, most vaccines will have to generate immunity for longer than the year and a half achieved by such vaccines to date. Even if long-lasting protection is not achieved, some vaccines, such as Levine's cholera vaccine, may be appropriate as short-term protection for travelers.

And although they do away with needles, mucosal vaccines face their own set of practical difficulties. Oral vaccines are difficult to give to infants, because babies don't swallow on command or may spit up. And if they are still breast-feeding, antibodies in the mother's milk can pick off potential vaccines before they can produce an immune response. "These are all things that have to be looked at," says Clements.

But in spite of these obstacles, there's increasing interest in the search for effective mucosal immunity. "I think there is going to be an explosion of knowledge in the next 6 years on delivering vaccines without needles," says Barry Bloom, an immunologist at the Albert Einstein College of Medicine in New York City. And many in the field hope that the effects of the blast, and the protection of the vaccines, lasts for a long time.

–Robert F. Service