A Vaccine for All Seasons

Genetic engineering is remodeling the smallpox vaccine to provide immunity against many other diseases

By JULIE ANN MILLER

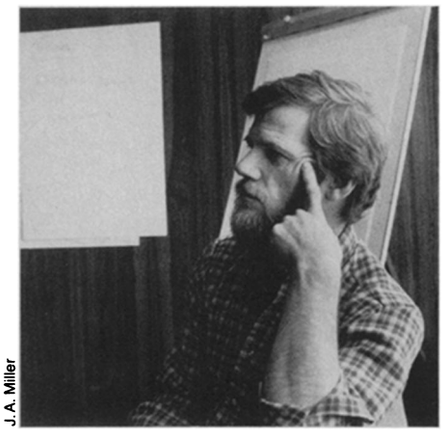
Vaccinia virus is the champion of immunizations. It makes up the vaccine responsible for the worldwide eradication of smallpox, an unprecedented achievement in preventive medicine. But since its triumph over smallpox, vaccinia virus has not been permanently retired from combat. With recombinant DNA methods, scientists are attempting to retool the live viral vaccine to fortify people and animals against a variety of diseases, ranging from influenza to malaria.

"Clinical trials have not yet started, but animal tests seem highly encouraging," said scientists in a summary report at the recent Dahlem Workshop on Biotechnology, held in West Berlin. "The potential ... seems overwhelming."

"Live recombinant vaccines are a blend of two arts," said Enzo Paoletti of the New York State Health Department laboratories in Albany. The "old art" is the attenuation of natural disease-causing agents to create an organism that produces immunity without disease. The "new art" is the strategy now being used to develop many vaccines: the identification and laboratory production of specific components of a pathogen that can create immunity, even without the rest of the infectious agent. Some such "subunit" vaccines, including hepatitis B vaccine and the vaccine against childhood meningitis, have already reached the market. But subunit vaccines do not reproduce in the body, so they require large amounts of material that may be difficult and expensive to produce. In addition, the body generally will mount only a weak immune response to an isolated chemical.

The blended strategy behind the recombinant vaccinia approach is to choose an immunity-creating component of a disease organism, as in the subunit vaccine approach. But instead of making this component in isolation, scientists snap its gene into the DNA of the vaccinia virus. The foreign gene then can be inserted in a location that disrupts a natural gene of the vaccinia, thereby further attenuating it. When a person or animal is inoculated with the remodeled vaccinia, a local infection results. The virus, carrying the foreign gene, replicates in the host cell and induces an immune response both to the vaccinia virus and to the microorganism that contributed the foreign gene.

Vaccinia, a large and rather complex virus containing about 200 genes, has an impressive capacity for carrying excess baggage. Scientists have calculated that the virus could accept several dozen foreign genes and still successfully infect cells and replicate. Thus, a single vaccine potentially could immunize the recipient against more than a dozen diseases. Scientists envision vaccinia vaccine customized by inserting "cassettes" of genes to meet the immunization needs of people or livestock with particular susceptibilities in a particular geographic area.



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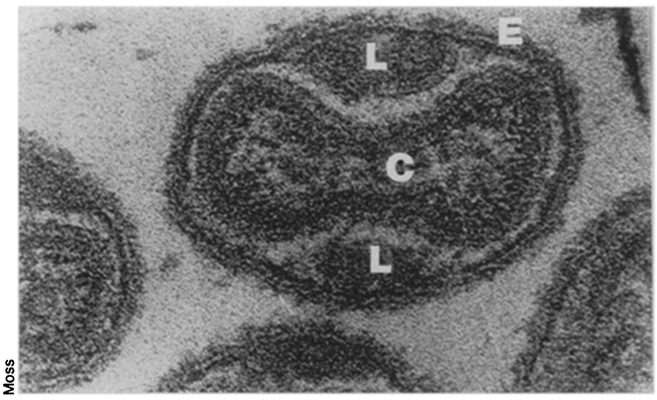


Moss

The vaccinia virus (left), which is about 300 nanometers long, is made up of a core (C) that contains the DNA and lateral bodies (L), surrounded by a protective envelope (E). Above: Paoletti at the workshop in Berlin.

Theoretically, many viruses could be used as carriers of immunity-provoking genes. (Indeed, there is one group of researchers working on inserting foreign genes into a herpes simplex virus.) But those scientists working on vaccinia argue that there is no need to look further. "Vaccinia has a history the others don't have," says Paoletti.

Their enthusiasm rests on the almost two centuries of experience with vaccinia for smallpox prevention. "The vaccine was inexpensive to produce," says Paoletti. "It could be stabilized as a freeze-dried preparation that could be shipped to all parts of the world in the absence of refrigeration. Administration of the vaccine did not require highly trained medical personnel nor sophisticated, expensive medical equipment." A genetically engineered vaccinia vaccine would be expected to have the same advantages.

The vaccinia virus is also particularly well suited for laboratory research because it has a very wide range of hosts, so potential vaccines can be tested on most laboratory animals. In fact, new vaccinia vaccines are expected to be developed for veterinary practice before any come into clinical use in humans.

In the recent research by Paoletti and by Bernard Moss and his colleagues at the National Institutes of Health in Bethesda, Md., more than half a dozen foreign genes have been inserted, and found to be active, in vaccinia virus. The research is most advanced in the case of a hepatitis B viral gene. There, Moss and colleagues have shown that chimpanzees inoculated with vaccinia containing the hepatitis B gene are no longer susceptible to infection by hepatitis B virus. In experiments aimed toward a veterinary vaccine against vesicular stomatitis, a contagious disease of horses, cattle and pigs, a gene from the disease-causing virus was in-

This is the first of a series of articles to appear over the next several months on current developments in biotechnology.

serted into vaccinia virus and used to protect cattle against infection.

In tests on small laboratory animals, other genes moved into vaccinia have given protection against influenza virus, herpes simplex virus and rabies virus. Moss reports in the May 10 SCIENCE that the herpes simplex-vaccinia virus is the first genetically engineered vaccine shown to protect against a latent infection, which normally persists for life, reactivating periodically. A gene from the malaria-causing organism has also been inserted into a vaccinia virus and shown to trigger antibody production when inoculated into laboratory animals.

"What we actually have is a delivery system," Moss says. The scientists believe that, with the appropriate protocol, any gene can be moved into vaccinia. When asked whether his group was working on a vaccine against the virus associated with acquired immune deficiency syndrome (AIDS), Moss said, "We'd be foolish not to be."

The construction of a genetically engineered vaccinia virus involves three steps: the selection and preparation of the foreign gene, the insertion of the gene into the virus and the purification of the altered virus.

When choosing a gene to put into vaccinia, genetic engineers rely on immunologists and clinicians to tell them which of a microbe's components best trigger host production of protective antibodies. "We will probably need to learn more about all viruses to determine which genes would be most useful to express," Moss says.

For viral diseases, the effective immunity-stimulating components are often found on the surface of the infectious agent. Thus, the gene for hepatitis B surface antigen was inserted into the vaccinia virus to create a hepatitis-fighting vaccine; the anti-herpes vaccinia was constructed with the gene for a herpes surface molecule called glycoprotein D; and the influenza-vaccinia recombinant vaccine contains the gene for influenza hemagglutinin.

Once a gene has been chosen, the trick is to disguise it to vaccinia as a vaccinia gene. The scientists do this by using control regions found adjacent to natural vaccinia genes. A carrier ring of DNA, called a plasmid, is assembled containing the foreign DNA, a promoter (the control region) and fragments of vaccinia DNA to direct the DNA to a specific location on a vaccinia virus.

In the second step of vaccine construc-

WHENCE VACCINIA?

Surprisingly, the origin of the vaccinia virus, used so long as a smallpox vaccine, is unknown. For many years most physicians believed that it was a form of the virus that causes cowpox, a disease of cows and other animals that is sometimes transmitted to farmworkers in contact with diseased animals. Indeed, most of the first vaccines came from cows or people infected from cows. "The Cowpox protects the human constitution from the infection of the Small-pox," wrote British physician Edward Jenner, who is generally credited with the introduction of vaccination in 1796. But recent analyses of the vaccinia virus show that it is clearly distinct from at least the modern cowpox virus. There is an indication that vaccinia may be related instead to a form of horsepox present in Jenner's day but now extinct.

Part of the confusion over poxes comes from the names. According to Derrick Baxby of the University of Liverpool, cows are not the primary reservoir of cowpox. It was named cowpox because the first strain to be characterized was isolated from a farmworker who had been infected while handling infected cattle. Baxby suggests its reservoir is some as-yet-unidentified small wild mammal, and cattle and humans only occasionally become infected.

Jenner and other early vaccine experimenters used, in addition to cowpox virus, material from an infection of horses. This infection, which Jenner called "grease" but which is more accurately known as horsepox, could also infect cows and humans. Thus some cows with pox may have been suffering from horsepox instead of cowpox. Virus from a horse, or from a cow infected by virus from a horse, was used successfully to inoculate people against smallpox. Although two other infections in horses today are colloquially called horsepox, the last outbreak of genuine horsepox occurred around 1900. Because horsepox was always relatively rare in horses, Baxby suggests that its true reservoir, like that of cowpox, may be in a small wild animal.

In contrast to cowpox and horsepox, vaccinia virus has no natural reservoir. It is maintained in laboratories for research and vaccine production. While it has been detected in humans, cows and a few other species, in each case it had been introduced by a recently vaccinated person.

The pedigrees of the smallpox vaccines used in the worldwide eradication program are uncertain. In the mid-19th century new strains were introduced for vaccine use, because the older strains, propagated "arm-to-arm," were losing effectiveness. In 1838 the National Vaccine Establishment in England claimed it was using "the matter originally collected by Dr. Jenner 38 years ago," but left it unclear which of Jenner's strains (which included both cowpox and horsepox) was being used. Since that time the evolution of the vaccines has become even more unclear.

Baxby discusses in detail possible origins of vaccinia in a book called *Jenner's Smallpox Vaccine* (Heinemann Educational Books, London, 1981). He concludes that the smallpox and the cowpox viruses are so different from vaccinia virus that it is very unlikely that vaccinia evolved from either of them during the evolutionarily brief time it has been maintained for vaccination. Instead, he believes today's vaccines are more similar to some of those used in the 19th century—most likely those derived from horsepox.

"It has been said that the origins of vaccinia will never be known. This is true," Baxby concludes. "All that one can do is analyse the early literature in the light of present-day information."

—J. A. Miller

Courtesy National Library of Medicine

Initially, the smallpox vaccine was regarded by some people as contrary to the will of God. They argued that inoculation of material from a "brute animal" would impart bovine characteristics to human recipients, who had been created in God's image. James Gillray satirized this view in his caricature — The Cow Pox —which shows the administration of vaccine obtained "Hot from ye cow" and the consequent eruption of cows from the lesions.

tion, an animal cell in laboratory culture is infected simultaneously with the plasmid and with normal vaccinia virus. While the vaccinia DNA is replicating free of its envelope, the disguised foreign gene can insert itself into the vaccinia DNA at its target location. The novel DNA enters the pool of replicating DNA molecules and is eventually packaged into a live recombinant vaccinia virus.

A variety of methods have been used to identify the recombinant virus among the pool of normal vaccinia. Some of these methods rely on the expression of the foreign genes introduced; others rely on the loss of expression of a vaccinia gene that was disrupted by the insertion of the foreign DNA.

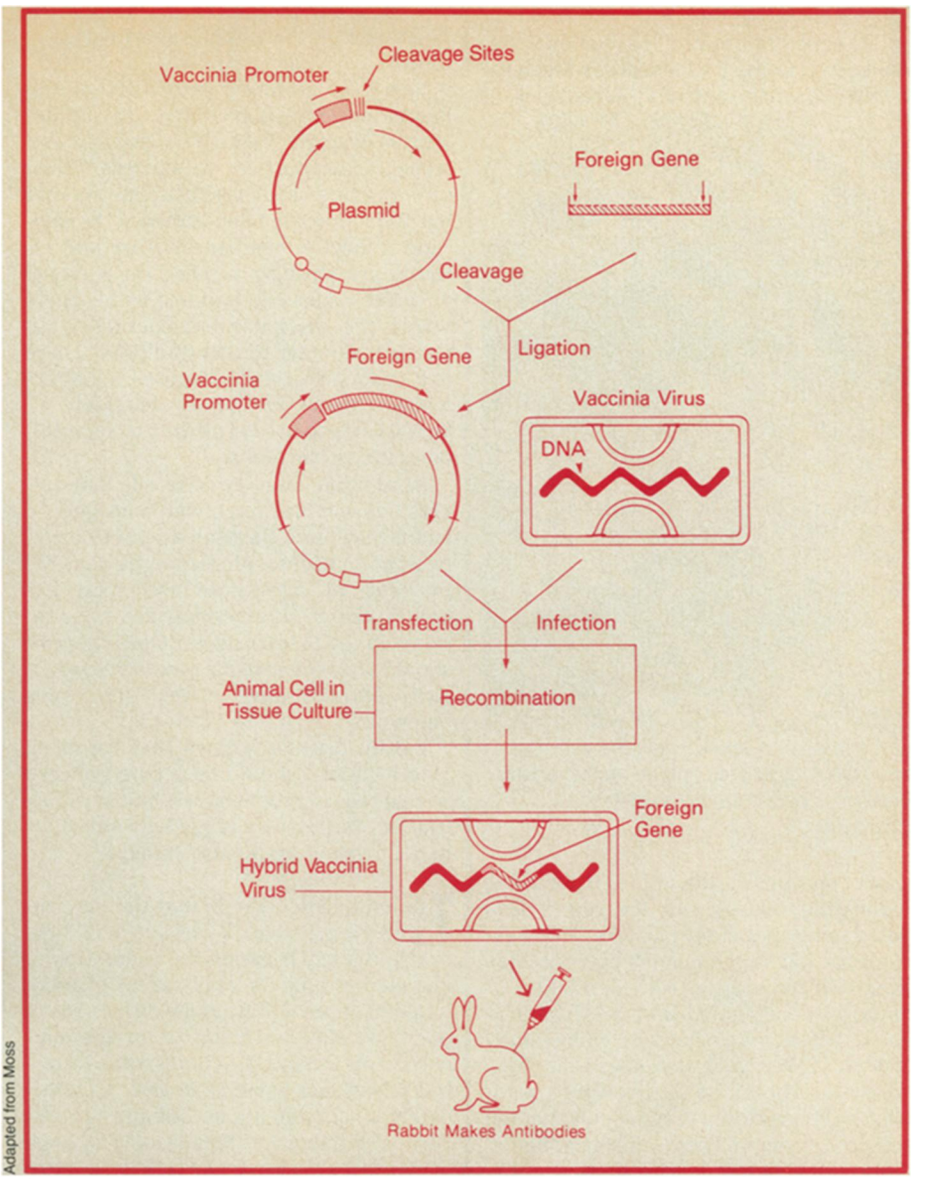
Jeffrey Fox

Moss

When the modified vaccinia virus is injected into a host, it successfully infects cells. The foreign gene does not seem to hinder the vaccinia's infectivity or subsequent replication. The foreign gene, like the natural vaccinia genes, directs the production of protein in the host cell. Most of the foreign genes that have been put into vaccinia thus far encode viral surface proteins. These proteins migrate to and insert themselves into the outer membrane of the host cell, where they trigger an immune response.

There is some concern that a foreign protein might be incorporated into the viral coat and change the infectious or growth characteristics of the virus. Though no evidence of this has been reported, Moss and his colleagues are looking further into this consideration.

Recombinant vaccinia viruses may be more effective in protecting against some infectious agents than against others. Animal experiments indicate promise for a wide range of diseases, including liver, nervous tissue and repiratory infections. "One area that has not been tested is diseases of the gastrointestinal tract," Moss says. It is likely that a vaccine against such diseases would require a new method of administration.



Adapted from Moss

In the remodeling of the smallpox vaccine, a gene of a different disease-causing organism is disguised as a vaccinia gene by attaching it to a stretch of vaccinia DNA called a promoter region. In animal cells in tissue culture, the foreign gene combines with the vaccinia DNA to produce a hybrid virus that can be used as a new vaccine.

Because the vaccinia virus is capable of carrying a large amount of foreign DNA, scientists are enthusiastic about creating a vaccine directed against a variety of infectious diseases. Paoletti has already inserted three different foreign genes into a single vaccinia virus. He reports that small laboratory animals inoculated with this polyvalent vaccine produce antibodies against all three foreign proteins — one from hepatitis B, one from herpes simplex and one from influenza virus.

"The question we have to answer ... is whether the immunological system can be saturated," Paoletti says. But he also raises the possibility of including in the vaccine elements that would provide a general stimulation to the immune system.

Once a person has received one vaccinia vaccine, could they be vaccinated successfully with another? The evidence so far is that the second response is not as strong as the first but still may be sufficient to produce immunity.

Paoletti says his research team has inoculated small animals with two different recombinant vaccinia vaccines, each containing a different foreign gene, at intervals of at least six weeks, and the animals produced antibodies against both foreign proteins. The second response was only about 10 percent as great as the first, but Paoletti suggests that use of stronger promoters could overcome this decrement.

Moss says that prior vaccination for smallpox should not limit the effectiveness of any new vaccinia vaccine. "Immunity to vaccinia wanes with time, and it has been many years since most people were vaccinated for smallpox," he says. Today's "young children [who would probably be the most important target group] have not been vaccinated for smallpox."

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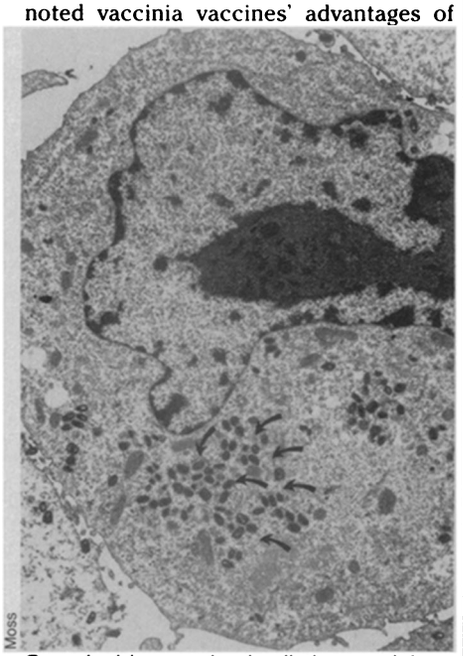
In a recent report, the National Research Council's Institute of Medicine noted vaccinia vaccines' advantages of



Once inside an animal cell, the vaccinia virus DNA replicates and is packaged into new virus particles (arrows) in the cytoplasm.

ease of production, low cost and use of live replicating viruses; but the report also noted some potential barriers. These include the inherent danger of potential side effects in individuals with dermatitis or immune deficiencies and uncertainties about when a vaccine has successfully "taken." The report also says that some countries might be reluctant to reintroduce the vaccinia vaccine now that the smallpox program has been dismantled.

Paoletti believes that the danger of side effects can be adequately reduced by employing standard medical screening practices to avoid vaccinating people with certain skin conditions or immune deficiencies.

A greater uncertainty, perhaps, is how the public will receive a live, genetically engineered vaccine. Some people consider it to be in the same controversy-provoking category as agricultural microbes that are to be released into the environment. Others argue that genetically engineered vaccines will be more predictable than the live vaccines already in wide use.

"To date, attenuation has been entirely empirical," says David J. Rowlands, who works on vaccine development at Wellcome Biotechnology Ltd. in Surrey, England. Viruses are just repeatedly grown in the laboratory until they become less virulent. Rowlands says the work on recombinant vaccinia virus by Paoletti and Moss is "one of the most exciting recent developments" in vaccine improvement.

According to Paoletti, "The wheel has been rediscovered." □

10 percent of NSF within a short period of time."

Larry W. Sumney, president of the Semiconductor Research Corp. in Research Triangle Park, N.C., pointed out some of the barriers to success. "Most universities are structured around discipline-oriented departments," he says, "and a faculty member's stature and rewards are strongly focused on personal achievements as determined by his peers within the discipline." Collaborative efforts run counter to that tradition. "There are case histories of faculty members whose careers have been adversely affected when they gave priority to such collaborations," he notes.

In addition, Sumney contends that universities are "not often well managed," a factor that could threaten a center's existence and success. Moreover, he says, 50 percent of academic research expenditures in engineering are concentrated in the 14 top schools. One objective should be to "elevate the research productivity of additional universities," says Sumney.

Finally, Sumney argues that "problem identification" should be a core concern so that research done is relevant to industrial needs. "History is rife with solutions to nonexistent problems," he says.

The optimistic view is that the new engineering research centers will help bridge the gap between the generation of knowledge and its application. "Today, fundamental scientific knowledge is one of our most effective forms of foreign aid," comments Roland W. Schmitt, chairman of NSF's National Science Board. "Unfortunately, it's foreign aid for our strong rivals — most notably, the Japanese." The hope is that these new centers will help strengthen the international competitive position of the United States.

For others, engineering research centers have another important role. "Imagine a medical school without a hospital," says one engineering professor. "That's the current position of U.S. engineering schools." Engineering research centers are the "hospitals" that may bring engineering research and education into the "real" world, he says.

"In some ways we are attempting to change the system, to push engineering research and education over a threshold into a new way of doing things," says H. Guyford Stever, president of the Universities Research Association in Washington, D.C. "So it is extremely important that we get it right from the beginning," says Stever, who chaired the NAS symposium.

Nevertheless, engineering research centers are still an experiment, and the results may not be known until well into the next century. "We don't expect all of these to be successful," says Nam P. Suh, NSF's assistant director for engineering. "Some are bound to fail." □