Gene research opens vaccine possibilities

Gene-splicing techniques hold promise for producing material for vaccines against infectious diseases. Significant steps toward such vaccines are being made, but the road will be long and arduous, said scientists at a recent meeting on molecular approaches to vaccine development at the National Institute of Allergy and Infectious Diseases. Richard Krause, director of NIAID, pointed out that although infectious diseases cause severe hardship world-wide, currently only about a dozen vaccines are in general use. Recombinant DNA techniques provide new opportunities for producing large amounts of pure viral components and also for imaginatively redesigning viruses for use in live vaccines, but progress is hindered by limited understanding of the immune system.

Among the advances reported were the production by recombinant DNA techniques of bacterial components implicated in diarrheal diseases and also of genes from influenza and hepatitis viruses. Bacteria making individual subunits of disease-causing toxins were engineered by Stanley Falkow of the University of Washington. The toxin of *Escherichia coli* has two subunits: One is the enzyme that produces the detrimental effect, the other is the delivery component, which binds to a gut cell so the toxic subunit can enter. Falkow has isolated the gene for the delivery component and attached it to bacterial DNA under the control of a well-understood bacterial regulatory element (the *lac* promoter). The bacteria containing the spliced DNA produce enough of the toxin subunit for thorough analysis. Falkow reports that the delivery unit of the diarrheal toxin closely resembles that of cholera toxin, with more than 85 percent of the amino acids being the same.

The delivery subunit of the toxin may be valuable vaccine material, since it should induce an immune response without harming the recipient. Falkow suggests that the same material may also work to immunize against cholera. The scientists also have bacteria making only the enzyme subunit without the delivery component. "We will try to 'defuse' it to some extent," Falkow says. In addition, they are examining a gene normally on a second ring of bacterial DNA that makes an attachment protein, which also must be present for severe diarrheal disease.

While vaccines are not yet available, the recombinant DNA is already providing a tool for what Falkow calls "epidemiology at a fine level." Now radioactive DNA containing specific genes can accurately identify in field tests bacteria containing the toxin and attachment genes.

Imaginative redesign of viral genes and their products is a vaccine approach being considered enthusiastically. In general, vaccines made of live viruses have been more effective and long-lasting than vaccines composed of a purified viral component. Scientists, however, do have ideas for improving effectiveness of purified material. Purnell Choppin of Rockefeller Univeristy is incorporating viral components into artificial membranes before inoculating. Daniel Nathans of Johns Hopkins University suggests that as scientists learn more about the immune response, they will be able to design new molecules to better induce immunity. "The synthetic techniques are essentially available. What we don't know is what we need to make," he says.

To use live viruses in vaccines, researchers must find genetically impaired viruses able to trigger a recipient's immune response, but not able to cause disease. One current approach to the subtle balance between infection and disease is to select viruses that cannot function at temperatures above a certain level. These viruses should survive and trigger an immune response in the upper respiratory tract, but they cannot survive in the warmer lower tract where they cause disease.

The worry with live vaccines is that some of the impaired virus's descendants will spontaneously correct their flaw and once again be able to produce disease. "There is an extraordinary ability of viruses to change," says Bernard Fields of Harvard Medical School. Ching-Juh Lai of NIAID agrees: "Genetic instability of attenuated genes is a formidable problem" The scientists described two ways by which an impaired virus could become virulent. The desirable alteration could change back to the original form or another change elsewhere in the genetic material could mask the flaw. Fields has observed that of viruses that lose their temperature-sensitive characteristic, approximately half revert to the original form and half accumulate further genetic alterations.

Scientists now are anticipating using new techniques to make deliberate changes, unlikely to revert, in viral genes. The first step, the reproduction of individual genes in rings of bacterial DNA, has already been accomplished for influenza virus, Lai reports. He has introduced genes from all three subtypes of human influenza into rings of bacterial DNA that reproduce in *E.coli*.He hopes to be able to construct stable, defined mutants by deleting portions of the genes and then converting them into the genetic material of functioning viruses. Malcolm Martin of NIAID says, "We now have the ability to introduce specific lesions at any part and make an attenuated virus with knowledge of how it has been changed."

A gene of hepatitis virus has also been reproduced in bacteria by researchers working with William Robinson at Stanford University. The gene codes for the surface protein that triggers an immune response. So far attempts have failed to force that gene to direct protein synthesis in bacteria. Dean Hamer of NIAID suggests that mammalian tissue cultured in the laboratory will be required for the expression of some genes. "I have a pessimistic suspicion that bacteria will not provide the ideal source of protein," Hamer says. Hamer described the technique by which rabbit and mouse genes for a blood protein were transferred into, and made to function in, monkey kidney cells in tissue culture.

Several of the scientists said that work toward various vaccines was being hampered by current NIH guidelines for recombinant DNA research. Nathans says, "I can't help but observe that tremendous progress has been made toward development of helpful vaccines since revision of the recombinant DNA guidelines in the last six months." He suggests that those regulations continue to be re-examined, not only with regard to changing views of the safety issues, but also with regard to experiments necessary to test promising vaccines. □

Rad waste sites closed

One after another, commercial radioactive dump sites are slamming their doors in the faces of producers of low-level wastes, forcing hospitals and universities — the major creators of such waste — to curtail cancer research and other medical experiments. The first door slammed last spring when Gov. Richard W. Riley of South Carolina placed a limit on the amount of wastes — such as radioactive liquids used in laboratory research or contaminated pipettes, film, cloth or other materials discarded after medical diagnostic tests that use radioactive isotopes — that would be accepted at the site in Barnwell. In July, Nevada's Gov. Robert List temporarily closed the Beatty site because of unsafe shipping practices. On Oct. 4, Gov. Dixy Lee Ray of Washington closed the third and last site at Hanford after badly packaged and leaking shipments arrived there. Eleven days later, Gov. Riley refused any more shipments from Commonwealth Edison Co. of Chicago, one of the firms responsible for the damaged shipments to Washington. This week, Gov. List closed the Nevada site permanently.

The "strike" has thrown universities and hospitals into confusion as to how and where to store their daily growing stocks of low-level wastes. (Not to be confused with much more highly radioactive wastes such as those from Three Mile Island, these are still often long-lived.) The actions result from the governors' dissatisfaction with the Nuclear Regulatory Commission's waste shipment inspection program. A meeting next month with the NRC may result in a stricter code. □