Vaccinia ‘Vector’ Vaccine Proves Protective Against Flu Challenge

With the advent of recombinant DNA techniques, the agent that eradicated smallpox from the world may one day serve as the vector for immunization against a variety of current major viral pathogens, including hepatitis B virus, influenza viruses, and herpesviruses. Such immunization might even be accomplished simultaneously, through the use of one polyvalent recombinant live virus vaccine. That is the picture emerging from vaccinia virus studies by Dr. Bernard Moss and co-workers at the National Institute of Allergy and Infectious Diseases.

In their latest study, reported in the Proceedings of the National Academy of Sciences (80:7155, 1983), the NIAID investigators found that, among other effects, a vaccinia virus recombinant containing a copy of an influenza A virus strain’s hemagglutinin gene raised specific antibody levels in hamsters and protected the animals against infection by that strain. This represents the first demonstration of actual protection against disease with a vaccinia-born “xenovirus” vaccine. Previous studies by the group had shown 1) that another hybrid vaccinia virus virus containing the gene for hepatitis B surface antigen (HBsAg) produced specific and potentially protective antibody levels in rabbits (see “Smallpox Conquered, Vaccinia Directed Against Hepatitis B,” HP, August 1983); 2) that the herpesvirus thymidine kinase gene could be cloned into and expressed by the vaccinia virus genome, as reported in the Proceedings of the National Academy of Sciences (79:7415, 1982); and 3) that the large size and relative lack of packaging constraints in the vaccinia virus genome permitted insertion and expression of a large amount of foreign DNA, which raises the possibility of constructing polyvalent recombinant vaccines whose added genes would induce immunity against several pathogens (Gene 25:21, 1983).

In view of the considerable worldwide morbidity and mortality associated with hepatitis B virus infection and the expense and effort needed to obtain and administer conventional hepatitis B vaccines, the NIAID group’s findings with a recombinant vaccinia vaccine expressing HBsAg are especially noteworthy. However, the experimental design did not permit viral challenge after inoculation because the animals used—rabbits—are not naturally susceptible to hepatitis B virus. Moss and co-workers are currently studying the efficacy of the recombinant vaccinia-hepatitis B vaccine in chimpanzees. Whatever the results of those studies, the demonstration that a recombinant vaccinia-influenza vacci-
cine is protective in other animals is a key step toward potential clinical use of recombinant virus vaccines.

As in the hepatitis B study, the influenza study (reported by Drs. Geoffrey L. Smith, Brian R. Murphy, and Moss) involved a two-stage procedure for construction of hybrid vaccinia virus. In the first stage, recombinant DNA techniques were used to assemble a plasmid containing the hemagglutinin gene of influenza virus A/Jap/305/57 (H2N2) flanked by nonessential segments of vaccinia DNA. In the second stage, this chimeric hemagglutinin gene was inserted into vaccinia virus by homologous recombination in cells infected with wild-type vaccinia virus and transfected with the chimeric gene-containing plasmid.

In tissue culture studies, cells infected with the purified recombinant synthesized influenza hemagglutinin, as demonstrated by specific antibody binding and autoradiography. Additionally, immunoprecipitation studies showed that the recombinant’s hemagglutinin polypeptide co-migrated with that immunoprecipitated from cells infected with wild-type influenza A/Jap/305/57. The investigators noted also that the recombinant’s hemagglutinin could be cleaved into two subunits when trypsin was added to infected cell cultures—a finding suggesting that the hemagglutinin was glycosylated and transported to the cell surface in much the same manner that is seen with its antigenically “authentic” counterpart.

Before testing the ability of the vaccinia virus recombinant to protect animals from influenza, Drs. Smith, Murphy, and (continued on page 44)
Respiratory: 
Central nervous system. 
Others. 
OVERDOSAGE: 
DURAPHYL 
Category C- Animal reproduction studies have not been conducted w1th 
whether theophylline can cause 
Pregnancy: 
potential or the effect on fertility of xanthine compounds 
to patients w1th congestive heart 
or more frequent doses 
Theophylline half-life 
hyperthyroidism, acute myocardial 
PRECAUTIONS 
General: 
cant change 
appreciated 
agon1sts were administered concomitantly, although not when either was administered 
Theophylline 
arrhythmias, convulsions or even death may appear as the first s1gn of 
patients. 
Reduction of dosage and laboratory 
Clearance has been documented in the 
with toxicity. The 
vs. 
associated with chron1c bronchitis and emphysema 
its components 
should not be 
DRUG 
Laboratory Test Interactions: 
When plasma levels of theophylline are measured by spectrophotometric methods: coffee, tea, cola 
beverages, chocolate, and ascorbic acid contribute falsely high values. 
Carcinogenesis, Mutagenesis, and Impairment of Fertility: 
Long-term animal studies have not been performed to evaluate the carcinogenic potential, mutagenic 
potential or the effect on fertility of xanthine compounds. 
Ovulation: 
Category C — Animal reproduction studies have not been conducted with theophylline. It is not known 
whether theophylline can cause fetal harm when administered to a pregnant woman or can affect repro 
and pregnancy. 
Xanthines should be given to a pregnant woman only if clearly needed 
Nursing Mothers: 
It has been reported that theophylline distributes readily into breast milk and may cause adverse effects 
the infant. Caution must be used in prescribing xanthines to a mother who is nursing, taking into 
account the risk/benefit of this therapy. 
PEDIATRIC USE: 
DURAPHYL is not generally recommended for use in children under six years of age. 
ADVERSE REACTIONS: 
The most consistent adverse reactions are usually due to overdose and are: 
Gastrointestinal: nausea, vomiting, epigastric pain, hematemesis, diarrhea. 
Central nervous system: headaches, irritability, restlessness, insomnia, reflex hyperexcitability, muscle 
weakness, convulsive and tonic generalized convulsions. 
Cardiovascular: palpitation, tachycardia, extrasystoles, flushing, hypotension, circulatory failure, ven 
tricular arrhythmias. 
Respiratory: tachypnea 
Renal: albuminuria, increased excretion of renal tubular and red blood cells, proteinuria, dysuria. 
Others: hyperglycemia and inappropriate ADH syndrome, rash. 
OVERDOSAGE: For information on management of overdosage, see full prescribing information. 
See full prescribing information before administering or prescribing.
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Day 40 was also the point at which influenza A/Jap/305/57 challenge was administered intranasally to all animals. One or two days later, the hamsters were killed so that their lungs and nasal turbinates could be removed, homogenized, and assayed on cell monolayers for influenza infection. Eight of the 10 animals inoculated with wild-type influenza virus as well as mice inoculated with vaccinia virus had detectable influenza in tissue homogenates, and the calculated mean tissue culture 50% infectious dose (TCID₅₀) per gram of lung tissue was approximately 4.4 in that group. In contrast, influenza A/Jap/305/57 was recovered from only two animals in the recombinant-inoculated group and from one animal in the influenza-inoculated group, and in all three the virus level was the lowest detectable (10³ TCID₅₀/gm of lung tissue). Moreover, the calculated mean quantity of influenza virus in both groups was only about 2.6 TCID₅₀/gm of lung tissue.

Commenting on their results, the NIAID investigators noted that the "high level of antibodies produced by hamsters vaccinated with the recombinant virus was correlated with resistance of those animals to challenge 6 weeks later with infectious influenza virus." They also pointed out that even though that resistance was derived from intradermal vaccination (on the animals' backs), it produced sufficient local immunity to rebuff an intranasal virus challenge. That result suggested that "vaccinia virus recombinants could be used as a vaccine to prevent infection with influenza or other respiratory viruses in man."

Referring to strain differences in influenza viruses, Drs. Smith, Murphy, and Moss observed that recent studies in their laboratory had shown that at least 25,000 base pairs of foreign DNA can be inserted into the 187,000 base pairs that make up the vaccinia virus genome. This flexibility would allow simultaneous use of different genes or multiple serotypes in a single vaccine. The investigators cautioned, however, that rapid antigenic variation in influenza virus infections still poses serious problems. "Although new vaccinia virus recombinants could rapidly be constructed," they noted, "frequent vaccinations would undoubtedly limit the intradermal growth of the recombinant and the production of antigen." The NIAID group is currently attempting to make high-expression recombinants so that sufficient antigen might be produced "after relatively few rounds of virus replication during secondary vaccinations."

On a more basic level, the recombinant vaccinia virus used in this study represents a valuable tool for further studies in animals, the investigators observed. As an immunization vector in animals, it retains the original vaccinia virus's infectivity and wide host range and at the same time elicits an influenza hemagglutinin-specific antibody response that is equal to or greater than that obtained after infection with live influenza virus. Because of those properties, they added, insertion of mutated influenza hemagglutinin genes into vaccinia virus should facilitate analysis of the genes' effects on both humoral and cell-mediated immunity. "This would seem to be a unique method of determining which regions of the HA molecule are important for an immune response during a live infection."