Considerations for Vaccines Prepared by Recombinant DNA Technology or Containing Recombinant DNA

The development of attenuated viral and bacterial strains for use as vaccines was one of the obvious applications of the techniques for recombining DNA discovered in the mid-1970s. Before this time, live viral (18) and bacterial (19) vaccine strains were developed by repeated in vitro passage or by chemical mutagenesis, techniques that resulted in undefined mutations. Nevertheless, live vaccines against diseases such as measles, mumps, rubella, and typhoid fever were developed and licensed. Recombinant DNA technology offered the means to develop attenuated vaccines in which the precise molecular mechanism of attenuation could be known.

Despite the precision of molecular DNA techniques, vaccines developed using recombinant DNA were thought by some to be threatening to the natural environment. To document the potential for environmental consequences of vaccinating humans with such vaccines, sponsors are required to include an environmental analysis (21 CFR 312.23), which includes justification for a claim for categorical exclusion or an environmental assessment. Such justification might include data showing the survivability of the vaccine strain in various natural environments such as local water, soil, and food, especially in comparison with the wild-type pathogen (20). Phase I protocols to study the safety of recombinant vaccines generally contain provisions for studying the potential for person-to-person transmission of these strains.

Considerations for Vaccines that Can Be Transmitted Person-to-Person

Many of the currently used live bacterial and viral vaccines are shed in respiratory secretions (e.g., live attenuated influenza vaccine) or stool (e.g., polio vaccine), and are potentially transmitted person-to-person (21). Transmission of oral polio vaccine was considered desirable in the early years of its use, since such transmission led to herd immunity (22). Transmission to pregnant women or immunocompromised individuals is now recognized as a risk of the use of live vaccines that can be spread from person-to-person, for example, transmission of vaccinia virus or its recombinant virus to an individual with eczema can result in severe vaccinia infection (23).

As a result of this concern, many phase I and II clinical protocols include preliminary measurements of the potential for person-to-person spread of live vaccine candidates. Initially, this may require that the vaccine strain be studied in isolation until a gross assessment of its transmissibility is established, for example, among unvaccinated adults residing with vaccinees on a research isolation ward. Examples of such studies to assess person-to-person transmission include studies of *S. typhi* vaccine strain CVD 908-*htrA* (24), *Shigella* vaccine strain 1208S (25), and of a recombinant vaccinia virus expressing gp160 of HIV (26). In phase II, volunteers who reside with infants, pregnant women, or immunocompromised individuals may be excluded because of the possibility of transmission of a vaccine strain whose safety is not completely established. phase II studies of transmissibility might include cultures of the stool or respiratory secretions of household contacts of vaccinees, and in later phases, attempts at vaccine isolation from environmental reservoirs such as sewage.

The testing of live oral cholera vaccine strain CVD 103-HgR is a good example of how such testing is executed. This *V. cholerae* O1 strain is deleted in 94% of the toxic A subunit of cholera toxin (27). In phase II clinical studies, the possibility of transmission of this strain to contacts of vaccinees and to the environment around the households of vaccinees was investigated (28,29). In brief, this strain was shed for a short period by only a small proportion of vaccinees, was minimally transmitted to contacts of vaccinees, and was not recovered from the natural environment near vaccinees.

SELECTION OF VOLUNTEERS

General Considerations

Initial phase I studies of candidate vaccines generally involve healthy adult volunteers, that is, those who have no abnormality that would confound the interpretation of the safety of the product or increase the likelihood of their having an adverse event. Healthy volunteers may be recruited from the community at large, or interested students, or employees at research institutions. Students and employees can be a vulnerable population, however, and care must be taken to ensure that there is no element of pressure or coercion to participate. In addition, some protocols may have a seroeligibility requirement, usually the absence of serum antibody to a particular antigen. Rarely, a protocol may specify that only individuals of a certain human leukocyte antigen (HLA) type may participate, when preclinical data indicate that immune responses will be restricted to a certain genotype. The protocol generally indicates what tests must be performed to establish volunteers' health. For example, some or all of the following may be done: medical history, physical examination, complete blood count, serum chemistries, urinalysis, HIV serology, and pregnancy test. Previously, women of childbearing potential were sometimes precluded from participation. However, in 1993, guidelines for the study and evaluation of gender differences in the clinical evaluation of drugs were issued, stating that women be included provided appropriate precautions against becoming pregnant are taken, and that women are counseled about the importance of these precautions. Efforts should be made to ensure that women participants are not pregnant at the time of enrollment, and that women are informed about animal reproduction studies and teratogenic potential of the vaccine. Generally, however, such data are not available for experimental vaccines. In 2001, the U.S. Department of Health and Human Services released additional protections pertaining to research in pregnant women. In these additional regulations, there must be direct benefit to the woman or fetus as a result of the research, or there must be only minimal risk to the fetus, and the new information learned in the research cannot be obtained in any other way.

Phase II vaccine studies also involve healthy adults. Once preliminary safety has been established in the phase I study, the screening to demonstrate the health of volunteers may be less rigorous. For example, the following may be done: medical history, complete blood count, HIV serology and pregnancy test.

The recent sequencing of the human genome has provided the opportunity to better understand the variations in safety and immune responses observed among apparently similar healthy individuals enrolled in vaccine trials. The importance of genotype on predicting response to drugs (pharmacogenomics) is well established, and these principles are beginning to be applied to understanding the variable occurrence of adverse events and immune responses to vaccines, so-called