Reports of enhanced antibody responses among elderly persons given thymosin-α1 and interleukin-2 suggest that addition of an immunomodulator may benefit selected populations (252–254). Dihydroepiandosterone (DHEA) has also been reported to have a modest enhancing effect (255). However, the growth factor GM-CSF failed to enhance serum antibody responses in healthy younger adults (256).

Formulations, Combination Adjuvants

Attempts to physically modify the vaccine to improve antigen presentation, such as formulation in liposomes (257,258), or multimeric complexes such as ISCOMs (immunostimulating complexes) (259,260). A liposomal vaccine formulated with IL-2 was more immunogenic than TIV among elderly subjects (261). Formulation of HA into ISCOMS with the adjuvant Quil A has resulted in improved antibody and CTL responses in phase I studies in humans (262,263). QS21, a saponin derived from Quil A, failed to augment antibody or cell-mediated responses in healthy adults when given with TIV, compared with TIV alone (264).

Virosomes (virus-like particles of 100–150 μ in diameter containing HA within the membrane) are approved for use in Europe, and are reportedly more immunogenic than standard inactivated vaccines (265). Structurally, virosomes are spherical vesicles of approximately 150 nm in diameter, composed of a lipid membrane with integrated envelope proteins derived from influenza virus, predominantly HA (266).

Because virosomes retain the cell binding and membrane fusion properties of the native virus, they are thought to interact efficiently with antigen-presenting cells, resulting in activation of T lymphocytes (267). In a recent study, a virosomal vaccine had similar immunogenicity as MF-59 adjuvanted vaccine in elderly, but lower rates of local side effects (268). Virosomal vaccine was also well tolerated and immunogenic in young children (269).

DNA Vaccines

Immunization of mice with DNA encoding the HA, as well as the internal M and NP proteins of influenza A, induces long-lived humoral and cellular immune responses (270,271), which are protective against viral infection and disease. Immunization of African green monkeys with DNA encoding a combination of three HAs and other influenza virus genes induced serum antibody against all three HAs (272). Most studies in humans of DNA vaccination for influenza have not shown impressive responses. However, epidermal delivery of DNA in the form of gold particles has been reported to elicit strong HAI antibody responses in humans (273).

Strategies to Enhance or Broaden Immune Responses to Other Viral Proteins

While the first priority for enhancing vaccine immune response is to increase antibody levels to the HA, optimizing responses to other viral proteins should improve protection against influenza. Because several of these epitopes (M, NP) are conserved between influenza A subtypes, vaccines based on these proteins offer the potential for increasing the breadth and duration of protection against diverse subtypes. Immunization with purified M2 protein has also been shown to ameliorate infections in animals (274), and vaccines based on the external domain of the transmembrane M2 protein, M2e, elicit cross-protective antibodies in a murine model (275,276). Because the immunity is less potent than HA-based immunity, and escape mutants emerge, it is likely that M2e will be used as an adjunct to current vaccines rather than as a stand alone antigen (277). Additional conserved determinants for protective antibodies are likely to exist (278). One such potentially cross-reactive epitope is the highly conserved maturational cleavage site of the HA(0) precursor of the influenza B virus HA, which has been shown to elicit a protective immune response to non-antigenically cross-reactive influenza B virus lineages (279).

CTLs are the principal mediators of recovery from pneumonia in the mouse model of influenza (280). CTLs likely are also important in hastening recovery and preventing pneumonia in humans. The main CTL targets in humans are conserved epitopes on the NP and M1 proteins (90). Recombinant DNA-expressed NP protein, plasmid DNA, and recombinant adenoviral vaccines induce CD8 CTL responses in mice that mediate protection against severe disease (270,281,282). Use of NP and M1 vaccines (purified proteins or plasmid DNA constructs) to expand memory cell populations for CTLs is under consideration.

Intranasal Inactivated Influenza Vaccines

Studies in humans conducted over many years have shown that nasopharyngeal administration of inactivated vaccines by nose drops or aerosol can stimulate production of local antibody in primed individuals (283–287). However, the simple administration of soluble antigen to this mucosal surface is inefficient, requiring relatively large amounts of antigen to induce mucosal immune responses. Research has therefore focused on ways to enhance the immunogenicity of mucosal inactivated vaccine by increasing uptake of antigen by mucosal antigen-presenting cells. Strategies used have included mucosal adjuvants, incorporation of HA and other antigens into particulate formulations, or both.

Bacterial enterotoxins, such as cholera toxin (CT), have been extensively evaluated as mucosal adjuvants for influenza and other vaccines. However, these toxins are far too reactogenic in man for routine use mucosally, as microgram quantities can induce cholera diarrhea if they reach the small intestine. Initial studies showing a potential adjuvant effect of purified B subunit (288,289) were complicated by the presence of residual amounts of holotoxin (290), and it became clearer that the holotoxin was responsible for the majority of the adjuvant effect (291). Further development has focused on engineering mutations designed to reduce or eliminate the diarrheagenic potential of CT or the highly related heat-labile toxin (LT) of enterotoxigenic *Escherichia coli,* while retaining adjuvanticity. Two types of mutations have shown promise: mutations that block the enzymatically active (ADP-ribosylating) site (e.g., LTK63 or LTR172) and mutations that block the protease activation site (e.g., LTG192) (292).

The largest experience in humans with intranasal inactivated influenza vaccine has been with HA formulated in phosphatidylcholine liposomes, and administered with fully enzymatically active LT. This vaccine was well tolerated in adults, children, and the elderly, and induced strong nasal anti-HA IgA responses in adults (293). Responses were less strong in the elderly, but the vaccine exceeded European licensing guidelines for serum antibody in all age groups (294). In addition, the vaccine was reported to be protective in adults and children, and to reduce the frequency of otitis media in otitis-prone children (295,296). However, this vaccine was later