also be used to boost Th1 responses primed by immunization with attenuated viral vectors. The concept of an attenuated viral vector prime followed by MF59 boost has been established in the clinic using canarypox vectors, as a strategy for both HIV (66) and CMV (67). Studies are also showing very encouraging preclinical data with alternative viral vectors, including alphaviruses and adenoviruses.

Future Perspectives on the Use of MF59

The encouraging safety and tolerability profile of MF59, in combination with immunogenicity data, suggest that MF59 is an appropriate adjuvant for use in pediatric populations. The stronger adjuvant effect of MF59 in comparison to alum in newborn infants immunized with an HIV vaccine has established the basis for further use of MF59 in this population. Moreover, preclinical data has firmly established that MF59 is a more potent adjuvant than alum for a wide range of vaccines, including recombinant proteins and protein polysaccharide conjugates. Moreover, if necessary, MF59 may be combined with various immune potentiators to enable the development of more complex vaccines, for example, against HCV, and/or HIV, which may also require the use of a prime with DNA or viral vectors.

VIROSOMAL VACCINES

An alternative antigen delivery system that is also included in a licensed influenza vaccine in Europe is called immunopotentiating reconstituted influenza virosomes (IRIV), or more simply ''virosomes.'' Virosomes represent a modification of an established drug delivery approach in which phospholipids are used to prepare vesicles, called liposomes, which have been used as delivery systems for a variety of entrapped drugs. For example, liposomes are used as delivery systems for anticancer drugs in marketed products. Virosomes are prepared by detergent disruption of influenza virus to free the viral membrane glycoproteins, followed by addition of phospholipids to allow vesicle formation, and removal of the detergents. Hence, the membrane antigens from influenza virus, mainly hemagglutinin, are presented in a particulate structure of similar size to the native virus. However, in contrast to the MF59 adjuvanted vaccine, which is focused on the elderly population who need an improved influenza vaccine due to immunosenescence, virosomal vaccines are used in all age groups. Virosomal vaccines appear to represent an alternative approach to inactivated whole virus influenza vaccines, which were originally introduced in the 1960s but have subsequently been largely replaced by subunit vaccines that are more highly purified and better tolerated. Although the virosomal influenza vaccines appear to be better tolerated than the original inactivated flu vaccines, there is limited evidence to suggest that they are actually more immunogenic than conventional influenza vaccines. When virosomal flu vaccines were directly compared with the MF59 adjuvanted vaccine, it was concluded that MF59 induced more potent immune responses (68). In addition, the safety profiles of virosomal and MF59 adjuvanted influenza vaccines appear to be comparable, with both showing only mild and transient local reactions at the injection site. Hence, while it is clear that MF59 offers a significant adjuvant effect for influenza vaccines, particularly for pandemic strains, it is less clear that the virosomal approach actually results in a more potent vaccine. Virosomes appear to offer an alternative means to deliver influenza antigens in a particulate structure that is well tolerated and can be administered to subjects of wide age range. There is significant interest in using the virosomal approach as a basic delivery system for a wide range of alternative vaccines (69), particularly for recombinant or peptide antigens that are poorly immunogenic when used alone.

POLYMERIC MICROPARTICLES FOR VACCINE DELIVERY

The adjuvant effect of synthetic microparticles has been known for many years and has been reviewed previously in detail (70). However, many of the kinds of particles used in early studies were nondegradable and consequently, were not appropriate for development for human use. In addition, since antigens were often chemically conjugated to the particles (71), this added a further level of complexity and made commercial development less likely. As an alternative approach, we have used microparticles prepared from biodegradable polymers with surface adsorbed antigens, since it has been demonstrated that organized arrays of antigens are able to efficiently cross-link B cell receptors and constitute a strong activation signal (72–74). In addition, studies have shown that the duration of antigen persistence is important in triggering protective T-cell responses (8), and antigen persistence is enhanced by microparticles, which offer protection against degradation in vivo.

The biodegradable and biocompatible polyesters, the poly (lactide-co-glycolides) (PLG), are the primary candidates for the development of microparticles as vaccine adjuvants, since these have already been used for biomedical purposes in humans for many years (75,76). In addition, PLG polymers have been used for the development of a controlled release drug delivery system for a therapeutic protein (77). Advantages of biodegradable microparticles for vaccine delivery include microparticle uptake ensures delivery of antigen into APCs; the multimeric array of adsorbed antigen epitopes on the surface of microparticles can enhance B cell interaction; microparticles are a flexible platform for co-delivery of immune potentiators and antigens; microparticles focus the effects of immune potentiators on immune cells, which may improve their safety profile; and biodegradable microparticles leave no residue in tissues. It has been shown on many occasions that particles of the appropriate size ($\sim$1 μm) are taken up efficiently by APC in vitro (78) and in vivo (79). Moreover, microparticles have been shown to be taken up by APC in vivo, which migrate to the T cell area of local lymph nodes and differentiate into DC (80). The physicochemical properties of microparticles, which control their uptake into macrophages include polymer hydrophobicity, surface charge, and particle size (81). It appears that cationic microparticles may be optimal for uptake into macrophages and DC (82).

PLG microparticles were first used for the delivery of entrapped antigens in the early 1990s (83,84). In addition to antibody responses, early studies showed that microparticles were able to induce CTL responses in rodents (85,86). This prompted speculation that microparticles may represent an attractive approach for the development of vaccines against tumors (87). However, the majority of early work focused on the use of microparticles for the controlled release of entrapped antigens, with the objective of making single dose vaccines (88,89). Single dose vaccines would be particularly advantageous in the developing world, where access to health care professionals is difficult to achieve. It was believed that controlled release of antigens from microparticles could be used to