aluminum-adsorbed vaccines do not show any advantage over soluble preparations for booster responses (51). While aluminum adjuvants can stimulate Th2 type responses in mice and the production of cytokines such as IL-4 and IL-5, as well as B cell production of IgG1 and IgE, they fail to stimulate Th1 responses such as IFN-γ production and B cell IgG2a secretion. The mechanism of adjuvanticity is still a subject of debate and includes formation of a depot at the injection site allowing slow release of antigen, stimulation of immunoreactive cells via activation of complement, activation of macrophages, and efficient uptake of aluminum-adsorbed antigen particles by antigen-presenting cells because of their particulate nature and optimum particle size (<10 μg) (16,51).

The limitations of aluminum adjuvants include (*i*) the potential for induction of occasional painful nodules or swelling and erythema at the inoculation site, and the induction of antigen-specific IgE antibody that correlates with such local reactions (51,52), although the incidence of systemic immediate hypersensitivity is probably less than one in a million (53). (*ii*) Aluminum has been detected at the site of subcutaneous injections for up to one year in animals (51), so it is not readily ''biodegradable.'' In addition, the aluminum compounds have several immunological drawbacks including (*iii*) their inability to enhance humoral immunity against certain vaccines in humans such as typhoid (54), influenza hemagglutinin antigen (55), and Hib capsular polysaccharide-tetanus toxoid conjugate (56), and (*iv*) their near total inability to elicit cell-mediated immune responses, particularly cytotoxic T-cell responses to intracellular organisms (16). Finally, (*v*) careful formulation of aluminum adjuvant preparations is required for reproducibility, they cannot be sterilized by filtration, and they cannot be frozen or readily lyophilized (51).

Microfluidized Oil/Water Emulsion (MF59)

A series of squalene emulsions were prepared using a microfluidizer to generate small particle (200–300 nm), oil-in-water (O/W) emulsions that had low-viscosity and were biodegradable (57). The most stable emulsion, termed MF59, consists of 4.3% (vol/vol) squalene and 0.5% (vol/vol) each of the surfactants Tween 80 (polyoxyethylene sorbitan monooleate) and Span 85 (sorbitan trioleate). Overall, MF59 generates antibody titers consistently higher than those obtained with aluminum hydroxide, equal to or higher than IFA, and equal to or lower than CFA, although it does not stimulate antibody responses against squalene (58). Results of timed injection studies suggest that MF59 microdroplets activate the immune system in the absence of antigen. It is postulated that macrophage uptake of the emulsion droplets results in cytokine production, which leads to an enhanced immune response in the presence of the antigen (57). MF59 has been tested in a variety of animal species, showing a good safety profile and a significant increase of the immune response to several subunit antigens including CMV, HSV, HIV, HCV, HBV, and influenza antigens.

Novartis Vaccines (formerly Chiron Biocine, Siena, Italy) registered an influenza vaccine adjuvanted with MF59 as *FLUAD*™ in much of Europe, which has been given to more than a million people (59). The MF59 formulation has also been tested in combination with pandemic influenza antigens, recombinant HSV glycoproteins, hepatitis B virus PreS2/S antigens, and HIV envelope proteins with various degrees of success (22). Study populations have included healthy adults (HSV, HBV, HIV, influenza) (60), elderly populations (influenza) (61), and infants and children (HIV) (57). Overall, MF59 has had acceptable reactogenicity profiles, although in the NIH comparison trial of multiadjuvanted HIV gp 120 vaccine described above, MF59 + MTP-PE (in addition to SAF + threonyl-MDP) induced significantly more moderate to severe local reactions than did other adjuvants (62).

Virosomes

Immunopotentiating reconstituted influenza virosomes (IRIV) are 150 nm unilamellar vesicular proteoliposomes composed of influenza H1N1 surface glycoproteins intercalated in a mixture of natural and synthetic phospholipids (63). The influenza HA antigen binds to sialic acid on the surface of antigen-presenting cells that take up the particles by receptor-mediated endocytosis and, subsequently, by pH induced membrane fusion with the phagolysosomal membrane. IRIV can act as antigen carriers to deliver many types of antigens bound or conjugated to the surface or internalized. Given the unique properties of the system, after proteolytic degradation, the antigenic peptides can become complexed with both MHC class I and class II molecules to be expressed on the surface of the APC.

The initial application of this system was with a virosomal hepatitis A vaccine. Berna Biologics, Ltd. (now owned by Crucell, Leiden, The Netherlands) registered *Epaxal*™ in several European, Asian, and South American countries after clinical testing, which showed an acceptable immune response and a significant reduction in local reactions compared to the conventional alum-adsorbed vaccine (64,65). A second example, *Inflexal V*™ (Solvay Pharmaceuticals, Brussels, Belgium), is a trivalent influenza vaccine that is made by mixing three monovalent virosomes, each one containing the seasonal HA and NA glycoproteins recommended annually by WHO (63). This technology was licensed by Solvay, and their virosomal influenza vaccine has been marketed as *Invivac*™ since 2004, showing similar immunogenicity to *FLUAD* and decreased reactogenicity (66). The virosome system is being further developed for use with a DPT vaccine, as well as other antigens.

Exotoxins

The bacterial ADP-ribosylating exotoxins (bAREs) represent a potent group of proteins that have been studied as enteric, nasal, and topical adjuvants for decades, and this category includes both licensed (albeit since withdrawn) and experimental vaccines. The only licensed vaccine that included a bARE as adjuvant was the intranasal virosome-based influenza vaccine that included a low dose of the *E. coli* heat-labile toxin (LT) for mucosal immunization (61,67). In pre-licensure trials, the vaccine was well-tolerated and elicited secretory IgA mucosal responses to influenza hemagglutinin, as well as serum antibody responses (68,69). However, post-licensure surveillance indicated that the vaccine was associated with an increased occurrence of Bell's palsy, and it was concluded that the intranasal administration of wild-type LT was likely to be an important contributing factor (70,71). Interestingly, extensive preclinical toxicology studies did not predict such adverse reactions (72).

AS04

GSK Biologicals has been developing novel AS for more than a decade. These are unique combinations of different compounds with immunomodulating abilities that can tailor an