action of many adjuvants. This is beginning to allow for rational design and combination of the optimal adjuvants for a particular antigen in a specific population, which can lead to safer and more effective vaccines.

SAFETY

During the past 75 years, many adjuvant compounds have been studied, but most were never accepted for routine vaccination because of their immediate toxicity and fear of delayed side effects. The current attitude regarding the risks and benefits of vaccination favors safety over efficacy when a vaccine is given to a healthy population of children and adults (30). In high-risk groups, including patients with cancer and AIDS, and for therapeutic vaccines, an additional level of toxicity may be acceptable when the benefit of the vaccine is substantial. However, the absolute safety of any vaccine cannot be guaranteed, so the risks must be minimized. Undesirable reactions can be grouped as either local or systemic.

The most frequent local adverse effects of vaccination are tenderness and swelling, with the most severe ones involving the formation of painful induration and nodules at the inoculum site. The mechanisms for such severe local reactions include formation of inflammatory immune complexes at the inoculation site by combination of the adjuvanted vaccine with preexisting antibodies resulting in an arthus-type reaction. In some cases, poor biodegradability of the adjuvanted vaccine may result in prolonged persistence in the tissues and reactive granuloma formation. Such local reactions are of concern for depot-type adjuvants and living vectors such as BCG. Those adverse effects are rare events with today's vaccines. Clinical studies typically reveal this type of problem before a vaccine is licensed and development is halted. Severe local reactions in humans followed subcutaneous injections of incomplete Freund's adjuvant (IFA), a mineral oil emulsion, using early formulations made with a mannide monooleate stabilizer that contained free fatty acid impurities. However, these lesions did not occur with IFA injected intramuscularly, and that contained the stabilizer without impurities (reviewed in Refs. 28 and 31). IFA has been administered to more than a million people worldwide (31–34). Despite the apparent long-term safety of this adjuvant (35), the risk/benefit ratio is felt to be too high for commercial use.

To date, vaccine adjuvants have caused few severe acute systemic adverse effects. More theoretical risks include the induction of autoimmunity or cancer. Fortunately, in 10-, 18-, and 35-year follow-up studies, the incidence of cancer, autoimmune and collagen disorders in 18,000 persons who received the IFA adjuvanted influenza vaccine in the early 1950s was not different from that in persons given aqueous vaccines (32,35–37). Autoimmunity can be triggered by an infection through either specific or nonspecific mechanisms, although this has been associated with vaccination only in rare circumstances, such as when a form of Guillain-Barré syndrome was linked to the 1976 to 1977 vaccination campaign against swine influenza (38). Extensive epidemiological studies have failed to show an association of autoimmune disease with vaccination in nearly all instances (39). Studies in animals can provide signals that would lead to further study. Anterior chamber uveitis has been reported with MDP and several MDP analogues in rabbits (40) and monkeys (41), and has been systematically sought in at least one adjuvant vaccine study involving 110 volunteers, but was not detected (42). Adjuvant-associated arthritis (43,44) has not been reported in humans, even after long-term follow-up (33,35,45). Anaphylactic reactions, angioedema, urticaria, and vasculitis have been described following the administration of the majority of vaccines, although severe events are rare (29). Finally, a syndrome known as macrophagic myofasciitis (MMF), characterized by diffuse arthromyalgias and fatigue in connection with muscle infiltration by macrophages and lymphocytes, has been described in France (46), although a causal association with vaccination has not been established.

REGULATORY ISSUES

In concert with the progress of the International Conference on Harmonisation (ICH) of technical requirements for registration of pharmaceuticals for human use, worldwide regulatory guidance on the development and testing of vaccines has expanded significantly in the past few years. Documents covering nearly every aspect of drug and biologic development are being created and revised in an effort to enhance and standardize the quality, safety, and efficacy of pharmaceutical products (http://www.ich.org, http://www.fda.gov/cber/guidelines.htm, and http://www.emea.eu.int). There is little advice directed specifically at the development of adjuvants in the United States, apart from their use in combination vaccines (47). The European Medicines Agency (EMEA) recently published a guidance that emphasizes a number of additional points: quality of manufacture of the adjuvant alone and in combination with vaccine antigens, nonclinical proof of concept and toxicity studies, and clinical development that assesses the adjuvant effect and dose required (48). It is important to note that as a rule, adjuvants are not licensed on their own. Since each combination of one or more antigens with an adjuvant has its own unique safety and efficacy profile, they are licensed and regulated as individual vaccine products in combination.

ADJUVANTS USED IN LICENSED VACCINES FOR HUMANS

Several adjuvants are licensed with their vaccines for human use in various parts of the world, including aluminum compounds, MF-59, virosomes, exotoxins, and AS04.

Aluminum Compounds

Aluminum salts, particularly aluminum hydroxide or phosphate, have been used for over 80 years (49) and have become the most widely used adjuvants in human vaccines. Vaccine antigens can be adsorbed to the amorphous crystalline gel by electrostatic interactions between proteins and the positively charged aluminum hydroxide. Alternatively, negatively charged aluminum phosphate gels can bind proteins through a ''ligand exchange'' between hydroxyl and phosphate groups (21). Calcium phosphate has also been used to adsorb DPT, inactivated polio vaccines, and allergens (50). The following licensed, parenterally administered human vaccines are combined with aluminum: diphtheria, pertussis, and tetanus alone or in various combinations with *Haemophilus influenzae* type b (Hib), inactivated polio, hepatitis B, hepatitis A, a rabies vaccine, an anthrax vaccine, and *Gardasil*™ a human papillomavirus vaccine recently licensed by Merck.

The major advantage of using aluminum adjuvants is their safety record after billions of doses, and the development of earlier, higher, and longer-lasting antibody after primary immunization compared to primary immunization with soluble vaccines, particularly of soluble toxoids, although the