Improving Vaccine Performance with Adjuvants

Frederick R. Vogel

New vaccines are presently under development and in testing for the control of infectious diseases, including human immunodeficiency virus (HIV) and tuberculosis. Several of these vaccines are composed of synthetic, recombinant, or highly purified subunit antigens. Subunit vaccines are designed to include only the antigens required for protective immunization and to be safer than whole-inactivated or live-attenuated vaccines. However, the purity of the subunit antigens and the absence of the self-adjuvanting immunomodulatory components associated with attenuated or killed vaccines often result in weaker immunogenicity. Immunologic adjuvants are agents that enhance specific immune responses to vaccines. Formulation of vaccines with potent adjuvants is an attractive approach for improving the performance of vaccines composed of subunit antigens. Adjuvants have diverse mechanisms of action and should be selected for use on the basis of the route of administration and the type of immune response (antibody, cell-mediated, or mucosal immunity) that is desired for a particular vaccine.

An immunologic adjuvant may be defined as any substance that, when incorporated into a vaccine formulation, acts generally to accelerate, prolong, or enhance the quality of specific immune responses to vaccine antigens. The word adjuvant is derived from the Latin verb adjuvare, which means to help or aid. Adjuvant mechanisms of action include the following: (1) increasing the biological or immunologic half-life of vaccine antigens; (2) improving antigen delivery to antigen-presenting cells (APCs), as well as antigen processing and presentation by the APCs; and (3) inducing the production of immunomodulatory cytokines. Through modulation of cytokine responses, adjuvant formulations can be designed that favor the development of T-helper type 1 (Th1) or type 2 (Th2) immune responses to vaccine antigens. Novel adjuvants are presently undergoing preclinical and clinical testing with human candidate vaccines, including experimental subunit vaccines against tuberculosis [1]. Standardized preclinical adjuvant-safety tests to support the clinical evaluation of novel adjuvants are also under development.

Immunologic adjuvants have been under development and in testing for most of this century. In the mid-1920s, Ramon [2, 3] observed that horses that developed abscesses at the site of an injection of diphtheria toxoid produced higher antitoxin titers than animals without abscesses. He later reported that abscesses induced by the injection of foreign substances together with toxoid also augmented antitoxin responses in horses. In 1926, Glenny [4] demonstrated the adjuvant activity of aluminum compounds with use of an alum-precipitated diphtheria toxoid vaccine.

In the mid-1930s, Freund [5] developed a powerful immunologic adjuvant composed of a water-in-mineral-oil emulsion and containing killed mycobacteria as an additional immunomodulator. This adjuvant is known as Freund's complete adjuvant (FCA), and although it is one of the most effective adjuvants known, it is highly reactogenic and cannot be used in human vaccines. However, Freund's incomplete adjuvant, which does not contain mycobacteria, was employed in an influenza vaccine licensed in the United Kingdom and is used in several HIV vaccines under clinical evaluation.

In 1956 Arthur Johnson discovered the adjuvant activity of endotoxins from gram-negative bacteria [6], and in 1974 Ellouz et al. [7] identified muramyl dipeptide as the smallest adjuvant-active component of the mycobacteria in Freund’s complete adjuvant. Presently, aluminum salt–based adjuvants continue to be the only immunologic adjuvants used in United States–licensed vaccines. However, hundreds of natural and synthetic compounds have been identified that have adjuvant activity. A number of these novel adjuvants, which may be used to augment or replace alum in human vaccines, have been under development and in preclinical evaluation for several decades [8]. In animal models, many novel adjuvants have been demonstrated to be more effective than alum in enhancing both antibody and cell-mediated immune responses to vaccine antigens. Extensive preclinical evaluation of novel immunologic adjuvants have been conducted, and clinical trials comparing the activities of various adjuvants have been initiated.

Advantages of the Use of Adjuvants

Potential advantages of the use of immunologic adjuvants in vaccine formulations include their ability (1) to direct and
optimize immune responses that are appropriate for the vaccine; (2) to enable mucosal delivery of vaccines; (3) to promote cell-mediated immune responses; (4) to enhance the immunogenicity of weaker immunogens, such as highly purified or recombinant antigens; (5) to reduce the amount of antigen or the frequency of immunization required to provide protective immunity; and (6) to improve the efficacy of vaccines in individuals with reduced or weakened immune responses, such as newborns, the aged, and immunocompromised vaccine recipients.

Types of Immunologic Adjuvants

Immunologic adjuvants can be classified by their sources, mechanisms of action, and physical or chemical properties. Table 1 lists examples of the types of adjuvants under development and in testing for use with human vaccines.

Adjuvant Mechanisms of Action

Adjuvants have diverse mechanisms of action and must be chosen for use with a particular vaccine on the basis of the route of administration to be employed and the type of immune responses desired. The first mechanism of adjuvant action identified was the so-called depot effect, in which gel-type adjuvants, such as aluminum hydroxide, or emulsion-based adjuvants, such as Freund’s incomplete adjuvant, associate with antigen and facilitate transport of antigen to the draining lymph node, where immune responses are generated. Immunogenicity of small antigens such as synthetic peptides that otherwise would be rapidly cleared from the injection site and from draining lymph nodes can be improved by the use of adjuvants that form particles or otherwise associate with and hold antigen.

Adjuvants can also act through enhancement of antigen presentation. Immunologic adjuvants act directly or indirectly on APCs, such as macrophages and dendritic cells [48, 49]. The emulsion-based adjuvant MF59 has recently been shown to be internalized by dendritic cells [22]. Certain novel adjuvants, such as purified saponins, immunostimulatory complexes, and liposomes, have been shown to greatly improve the induction of major histocompatibility complex (MHC) class I-restricted CD8+ cytotoxic T lymphocyte (CTL) responses over that induced by the same antigen given alone or in combination with standard alum adjuvants [33, 50–53].

These adjuvants may induce CTL responses by delivering antigen directly to the cytosol for presentation with MHC class I molecules [49]. Cytosolic antigen delivery by membrane-active adjuvants could mimic antigen presentation that occurs during viral infection or immunization with live-attenuated vaccines. Antigen presented to the cytosol could bypass endosomal antigen delivery and subsequent processing with MHC class II molecules, which occurs when antigen is delivered alone or in alum and induces primarily antibody responses [54] via presentation to CD4+ T helper lymphocytes. Adjuvants may also promote cytosolic antigen delivery and MHC class I presentation by enabling antigen to cross endosomal membranes into the cytosol after ingestion of antigen-adjuvant complexes by APCs [55].

Antigen can be targeted to macrophages or dendritic cells by particulate adjuvants such as liposomes. APCs can also be stimulated by adjuvants to secrete immunomodulatory cytokines. Various cytokines induced by adjuvants act on lymphocytes to promote predominately Th1 or Th2 immune responses [54, 56, 57]. Adjuvants that enhance Th1 immune responses through the induction of IFN-γ and delayed-type hypersensitivity also elicit the production of IgG subclasses that fix complement and bind with high affinity to Fc-γ-I receptors (e.g., IgG2a in mice and IgG1 in humans) [25, 58, 59]. These immunoglobulin subclasses are the most active in complement-mediated lysis and in antibody-dependent cell-mediated–cytotoxicity effector mechanisms.

Several cytokines are under evaluation as vaccine adjuvants, including IL-2, IFN-γ, granulocyte-macrophage colony stimulating factor, and IL-12 [43–46, 60]. IL-12 is a recently characterized cytokine that may play a pivotal role in the immunomodulatory activities of various immunologic adjuvants [61]. Jankovic et al. [47] showed that the addition of IL-12 to an alum-adsorbed HIV-1 gp120 vaccine elicited Th1 cytokines and IgG2 and IgG3 antibody responses in mice; the same vaccine without IL-12 induced Th2 cytokines and IgG1 antibody responses. Bacterial toxins with adjuvant activity, such as cholera toxin and pertussis toxin, which preferentially drive Th2-like

<table>
<thead>
<tr>
<th>Table 1. Types of immunologic adjuvants.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type, adjuvant</td>
</tr>
<tr>
<td>Gel-type</td>
</tr>
<tr>
<td>Aluminum hydroxide or aluminum phosphate</td>
</tr>
<tr>
<td>Calcium phosphate</td>
</tr>
<tr>
<td>Microbial</td>
</tr>
<tr>
<td>DNA CpG motifs</td>
</tr>
<tr>
<td>Cholera toxin</td>
</tr>
<tr>
<td>Escherichia coli heat-labile toxin</td>
</tr>
<tr>
<td>Pertussis toxin</td>
</tr>
<tr>
<td>Muramyl dipeptide</td>
</tr>
<tr>
<td>Oil-emulsion and emulsifier-based</td>
</tr>
<tr>
<td>Freund’s incomplete adjuvant</td>
</tr>
<tr>
<td>MF59</td>
</tr>
<tr>
<td>SAF</td>
</tr>
<tr>
<td>Particulate</td>
</tr>
<tr>
<td>Immunostimulatory complexes (ISCOMs)</td>
</tr>
<tr>
<td>Liposomes</td>
</tr>
<tr>
<td>Biodegradable microspheres</td>
</tr>
<tr>
<td>Saponins (QS-21)</td>
</tr>
<tr>
<td>Synthetic</td>
</tr>
<tr>
<td>Nonionic block copolymers</td>
</tr>
<tr>
<td>Muramyl peptide analogues</td>
</tr>
<tr>
<td>Polyphosphazene</td>
</tr>
<tr>
<td>Synthetic polynucleotides</td>
</tr>
<tr>
<td>Cytokines</td>
</tr>
<tr>
<td>IFN-γ</td>
</tr>
<tr>
<td>IL-2</td>
</tr>
<tr>
<td>IL-12</td>
</tr>
</tbody>
</table>
responses, have been shown to enhance IgA and IgE [12, 57, 62,63] antibody production. Adjuvants that drive Th2-like immune responses could enhance protection against mucosal virus transmission by augmenting IgA production.

**Adjuvant Safety**

The benefits of incorporating adjuvants into vaccine formulations to enhance immunogenicity must be weighed against the risk that these agents will induce adverse reactions. Local adverse reactions include local inflammation at the injection site and, rarely, the induction of granuloma or sterile abscess formation. Systemic reactions to adjuvants observed in laboratory animals include malaise, fever, adjuvant arthritis, and anterior uveitis [64, 65]. Such reactions often are caused by the interaction of the adjuvant and the antigen itself, or may be due to the type of response to a particular antigen the adjuvant produces, or the cytokine profile the adjuvant produces in an antigen. Therefore, even though separate and extensive preclinical toxicological and safety studies have been performed on both the adjuvant and the vaccine antigens, a final safety evaluation of the human candidate vaccine formulation proposed for phase I clinical testing should be conducted.

This evaluation should be conducted in a species of small animal in which the antigen has been found to be immunogenic and that can be reproducibly immunized by the same route proposed for the human clinical trials. The dose and frequency of immunization of the vaccine also should meet or exceed those anticipated for use in the clinical trial. Such a test, recently designed by a collaborative effort between the Center for Biologics Evaluation and Research/Food and Drug Administration and the National Institute of Allergy and Infectious Diseases, has been used to evaluate several vaccine formulations containing novel adjuvants [66].

**Future Directions**

Adjuvant research is a field that is advancing rapidly, which reflects the high rate at which new adjuvants are being discovered and the better understanding of immune mechanisms possible because of advances in immunobiology. In turn, adjuvants should now be applied to the study of many aspects of basic immunology. For example, adjuvants can be used as a tool to study immune mechanisms, such as antigen presentation by dendritic cells and modulation of immune responses by cytokines and their receptors. Adjuvants can also be employed in vaccine design research, which could assist in identifying the requirements of protective immunity, since different adjuvants vary immune responses to the same experimental antigen. The activities of adjuvants in humans as compared with their effect in small animals should be more fully evaluated. Animal models should be developed that can predict as accurately as possible the effectiveness in humans of a particular adjuvant when formulated with the desired vaccine antigens.

**Summary**

Development of safe and effective vaccines composed of subunit antigens will require the ability to selectively drive appropriate protective immune responses to them. The use of immunologic adjuvants to enhance and direct immune responses to subunit vaccines is a critical component of a rational vaccine design. Adjuvants have diverse mechanisms of action and must be selected for use on the basis of the immune responses (e.g., antibody, mucosal, and CTL) that contribute to the induction of protective immunity. Adjuvants can improve the performance of vaccines by targeting antigen to APCs, eliciting cytokines that direct Th1 or Th2 immune responses, promoting cell-mediated immunity (including CTL responses), and reducing the number of immunizations or the amount of antigen required for protective immunization.

The selection of a vaccine adjuvant should be based on analysis of the potential benefit of the adjuvant in enhancing the immunogenicity of a vaccine, weighed against its risk to induce adverse local or systemic reactions. The severity and prevalence of the disease against which the vaccine is designed to afford protection may also be considered in risk-and-benefit determinations for the use of novel adjuvants. Standardized methods to evaluate adjuvant safety should be implemented for human vaccines that are to be formulated with novel adjuvants.

**References**

10. Chu RS, Targon OS, Krieg AM, Lehmann PV, Harding CV. CpG oligo-
18. Mu HH, Sewell WA. Regulation of DTH and IgE responses by IL-4 and IFN-γ in immunized mice given pertussis toxin. Immunology 1994;83:639–45.
29. Richards RL, Rao M, Wassef NM, Glenn GM, Rothwell SW, Alving CR. Liposomes containing lipid A as an adjuvant for induction of an-


