chains of hydrophilic polyoxyethylene. A large number of copolymer adjuvants have been synthesized by varying the constituent chains (190). Nonionic block copolymers are currently used commercially in over-the-counter products, including shampoos, mouthwashes, and cosmetics. Copolymers are adhesive molecules that bind antigens to hydrophobic surfaces, such as oil drops or cells (191). Evidence suggests that proteins bound to copolymer are held firmly in a condensed fashion, and retain much of their native B-cell epitope confirmation when presented to macrophages and dendritic cells for immune processing (191). The activation of complement by contact with the copolymer surface augments the adjuvant effect. Several preparations of block polymers developed by Vaxcel, Inc. (Norcross, Georgia, U.S.) are awaiting clinical trial (190,192).

Cytokines

The use of cytokines as vaccine adjuvants has been encouraged due to a better understanding of cytokine mechanisms and the commercial availability of recombinant interferon-γ (IFN-γ) and granulocyte-macrophage stimulating factor (GM-CSF). Many cytokines (e.g., IL-3, IL-6, IL-11, GM-CSF) are capable of enhancing various immune responses when administered repeatedly. But the cytokines with the greatest potential are those administered in a single dose at or near the time of antigen injection; cytokines administered in this practical way include IFN-α, IFN-γ, IL-1, IL-2, IL-12, and GM-CSF. A cytokine can enhance, inhibit, or have no effect, depending on the dose, timing, and animal species, and which of these effects predominates is not always predictable (193,194).

The adjuvant effects of these cytokines in animals or humans have been reviewed in detail (195–197), although trial results to date have failed to document a strong adjuvant effect for cytokines in humans (198). The cytokine most intensively studied for its adjuvant activity is GM-CSF. It has been used with hepatitis B vaccine in patients with chronic renal failure (199,200) as well as in patients with HIV infection (201). In each of these studies, it enhanced the vaccine response, but was usually administered 24 hours before the vaccine. In contrast, when given concurrently with hepatitis A, influenza, and tetanus-diphtheria toxoid vaccines, a lower response was observed (202), which emphasizes that the effect of the timing of GM-CSF administration requires further study. Nevertheless, two meta-analyses concluded that the use of GM-CSF with hepatitis B vaccines both accelerated and increased the response rate (203,204). GM-CSF has also been used with a leishmania vaccine (128). Another cytokine, flt3 ligand, enhances dendritic cell numbers and function, but this effect was not translated into an improved antibody response (89,205). The use of rIL-12 with an experimental vaccine for CMV improved both humoral and cellular immune responses in human subjects (77).

Immunostimulatory Oligonucleotides: CpGs

Just as bacterial DNA can activate immune cells, synthetic oligodeoxynucleotides (ODN) containing unmethylated CpG dinucleotides in particular base contexts (CpG motifs) stimulate the innate immune system to induce protection in mice and primates (206,207). Either alone or in combination with a vaccine, they can activate human B cells, DC, and NK cells (208) and trigger an immune cascade that includes the production of cytokines, chemokines, and IgM to protect against infection. CpG ODN are extremely efficient inducers of Th1 immunity and CTL, and can allow a 10- to 100-fold reduction in the dose of antigen, presumably because of the increased efficiency of antigen presentation by DC (209). The administration of CpG ODN is currently being tested as a stand-alone treatment for cancer and asthma, and as a vaccine adjuvant (210–213). Acting through TLR9 receptors present on B cells and plasmacytoid dendritic cells, CpG has been shown in many studies to both accelerate and enhance the response to hepatitis B vaccines in human subjects (214,215) with protective and sustained levels of anti-hepatitis B surface antigen achieved with fewer doses of vaccine. The adjuvant improved the anti-HBs antibody levels in HIV patients (85), and enhanced the affinity of anti-HBs antibodies independently of the titers achieved (86). Immunostimulatory sequences have been used with hepatitis B vaccine proteins (87,214,216). While the addition of CpG ODN to hepatitis B vaccines has improved the immune response to this vaccine, no improvement was observed when added to an influenza vaccine (108). However, CpGs did enhance the response to that same vaccine when given at 1/10, the standard dose.

SUMMARY AND CONCLUSION

Every adjuvant has a complex and often multifactorial immunological mechanism, usually poorly understood in vivo, although the discovery of the TLRs and mechanisms of innate immunity will help guide adjuvant development over the next decade. Adjuvant safety, including the real and theoretical risks of administering vaccine adjuvants to humans, is a critical component that can enhance or retard adjuvant development. In addition to the problem of safety, several other issues impede the orderly development of adjuvanted vaccines. These include inconsistent immunopotentiation by candidate adjuvants, marked variation in response to the same adjuvant by different animal models, and the inability to consistently predict protective efficacy by immunoassays. However, decades of basic cellular research, preclinical experiments, and clinical safety and immunogenicity studies have led to a significant expansion in understanding the role of adjuvants in recent years. Hopefully, this will open new doors in vaccine research.

The most studied experimental adjuvants in man include aluminum compounds, oil-based emulsions with or without muramyl dipeptide, monophosphoryl (detoxified) lipid A, MPL, the triterpene glycoside QS-21, nonionic block copolymers, several cytokines, especially GM-CSF, and CpG ODNs. In preclinical studies of adjuvants and vaccines, depending on the antigen used, the same adjuvant can enhance, inhibit, or have no effect at all. The more important determinants of immunogenicity include the nature and dose of the immunogen, the schedule and route of administration, the population being immunized, the stability of the adjuvant formulation, and the choice of adjuvants used alone or in combination. The increasing understanding of these determinants is fundamental to the further development of new vaccines. This is beginning to allow for rational design and combination of the optimal adjuvants for a particular antigen in a specific population, which has the potential to lead to safer and more effective vaccines. In addition to immunologic enhancement without toxicity and successful protection against challenge, choice of adjuvant for a clinical trial may depend on cost and commercial availability. Rational development of classical and novel adjuvants will continue to be one of the most important challenges for the vaccinologist to be able to address persistent unmet medical needs.