results (50) and another showed that conjugation to the 5′-end of the ODN resulted in loss of adjuvant activity (51).

The second mechanism for synergy is to combine CpG ODN with another adjuvant that works on different cells or through different pathways. Such immune enhancement synergy has been demonstrated by combining CpG with monophosphoryl lipid A (MPL), a TLR4 agonist (43,44), QS21, a saponin immune modulator of unknown mechanism of action (52), or granulocyte-macrophage colony-stimulating factor (GM-CSF) (53).

DNA Vaccines and Viral Vectors: Role of CpG Motifs

DNA vaccines are plasmids naturally containing several hundred unmethylated CpG motifs, which appear to be necessary for adequate immunogenicity of the expressed antigen (54–56). It is possible to further enhance the immunogenicity of DNA vaccines by cloning in further CpG motifs (56) or coadministering it with additional noncoding vector DNA in mice (55,57–59) and primates (60,61). However, the addition of too many CpG motifs to a plasmid suppresses the humoral response, possibly because CpG-induced cytokines, such as type 1 interferons, suppress expression of antigen from the commonly used cytomegalovirus (CMV) promoter (61). It is also a delicate balance to add CpG ODN to a plasmid DNA vaccine because of the dose-dependent interference of the phosphorothioate ODN backbone with the uptake and expression of the plasmid (62,63). While gene gun delivery of DNA vaccines should avoid the cell uptake issues, immunization against an LCMV CTL epitope did not give increased levels of CTL after coating the beads with CpG ODN (64) that was delivered via gene gun. This may be due to cytokine downregulation of the plasmid promoter, or possibly the gene gun fails to deliver the CpG to the endosomal compartment where TLR9 is located. CpG ODN have also been incorporated into a live parvovirus vaccine. This combination resulted in enhanced immunogenicity and efficacy (survival from tumor challenge) in mice (65).

Prophylactic Infectious Disease Vaccines

The greatest body of CpG adjuvant work has been carried out with infectious disease antigens, where they have been shown in animal models, predominantly mice, to be very potent for augmenting humoral and cellular responses to an extensive list of antigens of viral, bacterial, fungal, or parasitic origin (Table 1). In some cases, challenge studies have also been carried out, with enhanced immunity usually correlating with increased protection (Table 1).

Interestingly, CpG ODN was able to enhance the immunogenicity of BCG vaccine and its efficacy against challenge with *Mycobacterium tuberculosis*, which presumably already contains immune stimulatory BCG-derived immunostimulatory CpG motifs (95). On the other hand, CpG ODN combined with *M. tuberculosis* culture filtrate proteins elicited enhanced IFN-γ responses but did not achieve protection against challenge with *M. tuberculosis* (111).

Therapeutic Infectious Disease Vaccines

The strong T_h1-like adjuvant effects of CpG ODN along with the ability to provide T help and to overcome hypo- and nonresponsiveness to antigens make it an ideal candidate as an immune enhancer in therapeutic vaccines to treat chronic infections. Likely target indications are chronic infections of hepatitis B and C where antigenic tolerance (HBV) and insufficient T_h1-type T-cell responses (HBV and HCV) are thought to contribute. There are no suitable small animal models for either of these diseases, but vaccinating with recombinant HBsAg and CpG ODN can break B- and T-cell tolerance in transgenic mice that express HBsAg protein principally in the liver under the control of the endogenous HBV promoter (112). Surprisingly, the resulting immune response clears circulating HBsAg and markedly reduces HBsAg mRNA expression in the liver without causing a cytopathic effect (113). Adoptive transfer experiments showed that both IFN-γ-secreting CD4 and CD8 T cells are responsible for the noncytolytic control of viral expression (114). Other chronic diseases that might benefit from treatment with a CpG-containing vaccine that would induce T_h1-type cell-mediated immunity include HSV, HIV, and TB.

Cancer Vaccines

The strong T_h1 adjuvant effects of CpG ODN make them ideal candidates to use with tumor antigens in cancer vaccines. The antitumor adjuvant properties of CpG ODN have been shown effective in various murine tumor models with several types of vaccines including (*i*) tumor-derived peptide in a melanoma model (115) and cervical carcinoma model (116), (*ii*) tumor-specific antigen in a B-cell lymphoma model (see below), (*iii*) tumor lysate in a glioblastoma model, (*iv*) irradiated whole-cell tumor vaccine in neuroblastoma (117) and renal cell carcinoma (RENCA) models (Weeratna and Davis, unpublished results), (*v*) idiotype of surface IgM in the 38C13 murine B-cell lymphoma model (118), (*vi*) an adenoviral vector expressing tumor-specific antigen in a prostate tumor model (119), (*vii*) pulsed DC vaccine in the RENCA model (120), (*viii*) DC cocultured with irradiated tumor cells in a murine colon cancer model (121), a live parvovirus vector (65), and (*ix*) adoptive transfer of T cells primed in vivo and restimulated ex vivo against the tumor cells in an A20 lymphoma model (122).

Allergy Vaccines

Allergic symptoms result from T_h2-type immune responses against otherwise harmless environmental antigens. T_h2 cytokines such as IL-4 and IL-5 induce B cells to secrete IgE, which in turn binds to high-affinity IgE Fc receptors on the surface of mast cells and basophils. If present, allergens can then bind to such surface IgE, cross-linking the IgE Fc receptors and leading to activation and degranulation of the mast cells or basophils. These cells release a variety of preformed proinflammatory and vasoactive compounds including histamine, prostaglandins, leukotrienes, and cytokines, resulting in an immediate inflammatory response that is often followed several hours later by a secondary reaction.

While antihistamines are effective for temporary control of allergic symptoms, research results have provided hope that CpG ODN, through induction of T_h1-type responses, could redirect the unwanted T_h2 allergic responses and provide a long-term or potentially permanent ''cure'' to allergic disease. Two basic approaches have been investigated, nonallergen-specific immune modulation (not the subject of this review) and use of CpG in allergy vaccines (123).

Studies in mice with previously established allergic disease (through repeated immunization with T_h2 adjuvants) have shown that vaccines containing low doses of allergens and CpG adjuvant induced T_h1-biased allergen-specific responses, reversing the established T_h2 responses and associated