CpG ODN to vaccines. Improved humoral and cellular responses are seen in piglets by using CpG ODN adjuvant with vaccines against *Toxoplasma gondii* (110), porcine reproductive and respiratory syndrome (37), and pseudorabies attenuated vaccine (128).

In cattle, addition of CpG ODN significantly enhanced T-cell and antibody responses to mycobacterial antigens and provided better protection against tuberculosis challenge (129). Similar enhanced immunogenicity and protection from challenge in cows were seen upon adding CpG to a bovine viral diarrhea virus vaccine (130) and a bovine respiratory syncytial virus vaccine (131,132). CpG adjuvant has also been shown to be effective in horses (133). At the lower end of the evolutionary scale, CpG ODN activate the immune systems of fish (134,135), which are commonly vaccinated within the aquaculture industry.

HUMAN EXPERIENCE WITH CpG ODN AS VACCINE ADJUVANTS

Prior to testing CpG adjuvants in humans, they had been tested for their ability to activate human lymphocytes ex vivo. Since such studies gave similar results to those with mouse splenocytes (136), it was expected that they would prove to be effective adjuvants in humans. Indeed, CpG ODN adjuvants have since been administered to several thousand humans with different antigens in clinical trials and have been shown to be highly effective and well tolerated.

Infectious Disease Vaccines: Clinical Experience

Immunogenicity studies have been carried out in humans with a variety of infectious disease antigens. Several of these studies have been carried out using HBsAg. In healthy volunteers, CpG ODN added to a commercial HBV vaccine containing alum adsorbed HBsAg resulted in significantly faster induction of antibodies that were of significantly higher levels (~10-fold) and avidity. Remarkably, with CpG added (0.5 mg), a single vaccine dose induced 75% seroprotection (anti-HBs $\geq$ 10 mIU/mL) compared with only 13% for the control vaccine (137,138). A similar study in HIV^+ hyporesponders who failed previous HBV vaccination showed significantly better humoral and T-cell (lymphoproliferative) responses when CpG was added to the commercial HBV vaccine (139,140), and this difference between groups was maintained even five years later (141).

In two other studies, a CpG ODN was administered with HBsAg alone (no alum). With the knowledge from animal studies that alum provides a benefit by binding the CpG and antigen together, the CpG effect would be expected to be weaker than that in the trials where an alum-based vaccine was used. Indeed, this was the case in one study, which used the same dose of CpG as had been used with the alum-based vaccines (142). However, in another trial, a higher dose of CpG was used with good results (143), and this latter vaccine, known as HEPLISAV™ [Dynavax Technologies (Berkeley, California, U.S.) and Merck & Co. Inc. (West Point, Pennsylvania, U.S.)], is now in phase III testing.

Results were also disappointing when CpG was tested with a single-dose, trivalent, split influenza vaccine; in this case, antibody titers were enhanced with CpG over control only when subjects had some preexisting immunity. It is likely that the poor results can be attributed, at least in part, to the lack of alum to hold the relatively low dose of CpG and antigen together (144).

CpG ODN has also been shown to be a highly effective adjuvant in healthy volunteers with other antigens when alum was included. Adding CpG ODN to BioThrax® [anthrax vaccine adsorbed (AVA)], a commercial *Bacillus anthracis* vaccine (that contains protective antigen along with other *B. anthracis* proteins), resulted in significantly higher peak antibody response and a three-week shorter time to attainment of a seroprotective antibody compared with AVA alone (91). In a phase I study of a *P. falciparum* malaria vaccine (AMA1-C1) with CpG ODN plus alum as adjuvants, an 8- to 10-fold increase in anti-AMA1 titers was observed versus alum alone (145).

Cancer Vaccines: Clinical Experience

CpG ODN has been utilized as an adjuvant with a number of tumor antigens in oncology patients. In these cases, measurement of T-cell responses is thought to be the best measure for potential efficacy. Strong antigen-specific T cells have been induced in melanoma patients by adding CpG to NY-ESO peptide (146,147).

Allergy Vaccines: Clinical Experience

As discussed above, the concept of CpG-containing allergy vaccines is to redirect previously existing symptom-causing T_h2 responses against an allergen into nonsymptomatic T_h1-type responses. TOLAMBA™ (Dynavax) is a vaccine comprising the Amb a1 allergen of ragweed conjugated to CpG. In phase I and II clinical trials in subjects with ragweed allergy, the TOLAMBA vaccine was shown to be well tolerated and to induce Amb a1–specific IgG but not IgE (49). However, a phase III trial failed to meet its primary end points, and the development program was stopped. The reason for the failed trial is unclear, although there were issues of placebo-treated subjects having lower than expected symptoms during the following ragweed season. Therefore, it is unclear whether this approach will one day be used for clinical benefit. There are those who believe that allergen-specific immunotherapy (i.e., allergy vaccines) will require a long treatment time with a large number of doses (148). If this is true, allergy vaccines will offer fewer advantages over classical desensitization therapy than originally hoped. Nevertheless, the possibility to reduce the risk of anaphylactic shock is not insignificant (124).

SAFETY OF CpG ODN

In the numerous animal models reviewed in the previous sections, CpG ODN has proven to be not only potent but also well tolerated across a wide range of doses. In comparative studies in animals, CpG has been shown to be less reactogenic in mice than other adjuvants (44). In marmosets, incomplete Freund's adjuvant with a *Plasmodium vivax* vaccine caused ulceration at the injection site, whereas CpG did not (106).

In humans, CpG ODN vaccine adjuvants have also been shown in several clinical trials with different antigens to be well tolerated when delivered by intramuscular or subcutaneous injection (138–147,149). Effects of the ODN backbone per se were not expected to pose problems, since chemically similar molecules had been given in doses of higher orders of magnitude and much more frequently in antisense trials.

Prior to testing began in humans, the greatest perceived risk with CpG ODN was that its strong T_h1 immune effects might induce autoimmunity, especially against DNA. Natural environmental exposures to CpG ODN in the form of infections are quite frequent and have not been shown to lead to an increased risk of autoimmune disease in humans, although