DNA, the Immune System, and Atopic Disease

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The prevalence and severity of atopic diseases (atopic dermatitis, asthma, and allergic rhinitis) have increased over recent decades, particularly in industrialized nations. Atopic dermatitis, like asthma, is more common in older siblings and in less crowded houses and with late entry to day care, increased maternal education, and higher socio-economic status. The inverse relationship between the incidence of atopy and childhood infections has led to the "hygiene hypothesis," which suggests that diminished exposure to childhood infections in modern society has led to decreased TH1-type responses. Reduced TH1 may lead to enhanced TH2-type inflammation, which is important in promoting asthma and allergic disease. Corticosteroids, commonly used to treat these conditions, inhibit the function of inflammatory cells, but they are ineffective in altering the initial TH2-type response to allergens in a sensitized individual. Treatment with TH1 cytokines not only has failed to make any significant impact on the outcome of these diseases, but it also has caused significant adverse reactions. A novel therapeutic approach, recently reported in the preclinical setting, is the use of oligodeoxynucleotides, which contain unmethylated motifs centered on CG dinucleotides. These CpG oligodeoxynucleotides potently induce TH1 cytokines and suppress TH2 cytokines, and can prevent manifestations of asthma and other allergic diseases in animal models. They have the potential to reverse TH2-type responses to allergens and thus restore balance to the immune system without the adverse effects of TH1 cytokines. ***Key words: atopic dermatitis/allergic rhinitis/asthma/DNA/oligodeoxy nucleotides. J Investig Dermatol Symp Proc 9:23–28, 2004***

Atopic eczema or dermatitis (AD) is often the first manifestation of atopy in a child at risk of developing atopic diseases. Allergic responses to food are similarly early, followed by asthma and allergic rhinitis (AR) (Holgate and Church, 1993). Approximately 80% of children with AD develop asthma or allergic rhinitis. These children frequently have more severe asthma than do asthmatic children without AD (Buffum and Settipane, 1966). Indeed, recent studies suggest that the immune mechanisms underlying asthma and AD have more similarities than differences (Leung, 1999). Despite an expanding repertoire of medications available for the treatment of asthma and other atopic disorders in the past three decades, the prevalence, severity, and mortality of asthma have increased significantly (Martin *et al*, 1996; Sears, 1991).

Predominant tissue eosinophilia is a hallmark of allergic inflammation (Martin *et al*, 1996). The number, activity, and survival of eosinophils are controlled through multiple pathways, including cytokines released by inflammatory cells such as T helper cells, NK cells, eosinophils, and mast cells. T helper lymphocytes can be divided into TH1 and TH2 cells on the basis on their cytokine production (Mosmann *et al*, 1986). TH1 cells produce IL-2 and IFN-γ (TH1 cytokines); TH2 cells produce IL-4, IL-5, IL-6, IL-10 ,and IL-13 (TH2 cytokines).

TH1 and TH2 cells interact in a counter-regulatory fashion, maintaining a critical balance. On the one hand, IL-4 promotes TH2 cell maturation from naïve TH0 cells (Swain *et al*, 1990) and suppresses TH1 cells and their cytokine production (Moore *et al*, 1990). On the other hand, IFN-γ inhibits the proliferation of TH2 cells (Gajewski *et al*, 1988) and promotes TH1 cells (Parronchi *et al*, 1992). Macrophage-derived IL-12 can swing the balance toward TH1 (Bliss *et al*, 1996), at least in part through the induction of IFN-γ (Micallef *et al*, 1996). The TH2 cytokines IL-4, IL-5, IL-9, and IL-13 (Sinigaglia *et al*, 1999) promote eosinophil production, recruitment, and survival; they are also important in the isotype switching of B cells to IgE (Nakajima *et al*, 1992; Ohnishi *et al*, 1993). In turn, allergen-specific IgE plays an important role in eosinophil recruitment during the allergic late-phase inflammatory response (Coyle *et al*, 1996). TH1-type responses can be induced and TH2 responses can be suppressed by exposure to pathogen-associated molecular patterns (PAMP), such as bacteria-like DNA, that contain CpG motifs. This review describes the studies supporting the use of CpG DNA in the immunomodulation of allergic inflammation (overview in **Table I**).

Current therapy for asthma is centered on anti-inflammatory agents, with corticosteroids the "gold standard" for treatment. These agents broadly reduce the inflammatory response (systemically as well as in the lungs), but they work "downstream" and have their major effects on effector cells in the lungs. They generally do not alter the initial antigen-induced immune response to allergens in sensitized individuals; there is debate over the extent of their effect on allergen-specific IgE; and they have no direct effect on suppression of TH2-mediated pathophysiologic changes. Indeed, there is some evidence that steroids enhance TH2 responses (Franchimont *et al*, 2000; Ramirez, 1998). Moreover, their toxicities (especially when administered systemically) are manifold, ranging from adrenal-cortical insufficiency to osteoporosis. The need clearly exists for novel disease-modifying pharmacotherapeutic agents for the treatment of asthma and other allergic disorders.

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Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; ODN, oligodeoxynucleotide; PAMP, pathogen-associated molecular pattern.

Table I. Effects of CpG ODN on animal models of the human allergic diseases

Disease model	Animal used	CpG ODN + Ag and route	Effects on physiology	Cellular inflammation	TH2 cytokines	TH1 cytokines	Antibodies and chemokines	Timing of treatment	Study
Asthma	C57BL/6 mice	SEA + ODN i.p.	↓ AHR	↓ Lung Eos. ↓ BALF Eos	↓ IL-4 in BALF	↑ IL-12 BALF ↑ IFN-γ BALF	↓ Total IgE	During sensitization	(Kline *et al*, 1998)
Asthma	BALB/c mice	Ova + ODN i.p.	↓ AHR	↓ Lung Eos. ↓ BALF Eos. ↓ Bone marrow	↓ IL-5 ↓ GM-CSF ↓ IL-3 (Splenocytes)	↑IFN-γ Splenocytes	Not done	One day before challenge	(Broide *et al*, 1998)
Asthma	BALB/c	Ragweed + ODN	↓ AHR	↓ BALF Eos.	↓ IL-4 BALF & splenocytes	↑IFN-γ BALF ↑IFN-γ Splenocytes	↓ Total IgE	0–48 hours before challenge	(Sur *et al*, 1999)
Asthma	BALB/c	Ova + ODN	↓ AHR	↓ BALF Eos. ↓ Lung Eos.	↓ IL-4 LN ↓ IL-5 LN		↓ Specific IgE	1 week before challenge	(Shirota *et al*, 2000)
Asthma	BALB/c	Ova + ODN covalently linked intra-tracheal	↓ AHR	↓ BALF Eos.	↓ IL-4 BALF ↓ IL-5 BALF ↓ IL-4 LN ↓ IL-5 LN	↑IFN-γBALF ↑IFN-γ LN	Not done	1 week before challenge	(Shirota *et al*, 2000)
Allergic conjunctivitis	SWR/J mice	Ragweed + ODN i.p or mucosal	↓ Clinical score ↓ Early and late phase response	Not done	Not done	↑IFN-γLN	↓ Specific IgE	3 days before or during challenge	(Magone *et al*, 2000)
Immunogenicity & allergenicity	BALB/c mice White Rabbit & Cynomolgus Monkey	Amb a 1 + ODN (conjugated) intradermal or i.p.	↓ Histamine release with conjugate but not with ODN	Not done	↓ IL-5 splenocytes	↑IFN-γ Splenocytes	↓ IgG1 ↓ Specific IgE ↑ IgG 2a	5,7, and 9 weeks after initial sensitization	(Tighe *et al*, 2000)
Asthma	BALB/c	Amb a 1 + ODN	↓ AHR	↓ BALF Eos. ↓ Lung Eos.	↓ IL-5 splenocytes	↑IFN-γ Splenocytes	↓IgG1 ↑IgG 2a	Day 14 and 21 after sensitization	(Santeliz *et al*, 2002)
Asthma	C57BL/6	Ova + ODN and SEA + ODN	↓ AHR	↓ BALF Eos. ↓ Lung Eos.	↓ IL-5 splenocytes	↑IFN-γ Splenocytes	↑ IP 10 ↑ RANTES ↓ Eotaxin	4, 6, 8 and 10 weeks after sensitization	(Kline *et al*, 2002)
Allergic Rhinitis	BABL/c	Ova + ODN	↓ Nasal scratching	↓ Submucosal Eos. ↓ Bone marrow Eos.	↓ IL-5 splenocytes ↓ IL-4 splenocytes	↑IFN-γ Splenocytes	Not done	At the time of sensitization	(Hussain *et al*, 2002)

Key: BALF = Bronchoalveolar lavage fluid, LN = Regional lymph nodes, AHR = Airway hyperreactivity, Eos. = Eosinophils, ODN = CpG Oligodeoxynucleotides,

THE HYGIENE HYPOTHESIS AND ITS PROBLEMS

One currently popular explanation for the rise of asthma prevalence and severity is the hygiene hypothesis. Data suggest that the increasing prevalence of asthma and allergic diseases in industrialized countries may be due to a lack of early childhood infections. Von Mutius and colleagues demonstrated a prevalence of asthma and allergic diseases in the former East Germany that is lower than in the former West Germany, despite worse air pollution and lower living standards in the eastern regions. This difference in prevalence was associated with early childhood infection in group day care settings in the East (von Mutius *et al*, 1992). The link between early infections and decreased incidence of asthma and other allergic diseases is further strengthened by family studies in which subjects with greater numbers of older siblings are relatively protected against the development of atopy and asthma; this may also be due to the earlier and more frequent occurrence of childhood infections induced by older siblings returning home from school or day care (von Mutius *et al*, 1994). More direct evidence on the role of microbial infections in protecting against asthma and atopy comes from a study carried out in Guinea-Bissau in 1979. Shaheen and colleagues demonstrated that a history of measles infection was associated with significant reduction in the risk of atopy (Shaheen *et al*, 1996). In related investigations, Shirakawa and colleagues found that the strength of positive tuberculin skin response was inversely associated with the incidence of asthma and atopy in Japanese school children (Shirakawa *et al*, 1997). Tuberculin response correlated with induction of TH1 cytokines (IFN-γ) and suppression of TH2 cytokines (IL-4 and IL-13).

All of these studies support the idea that early-life infections may protect against the development of atopy and atopic diseases like asthma. This so-called hygiene hypothesis has been given an immunological framework in which the balance between TH1-type and TH2-type immune responses is pivotal (Matricardi and Bonini, 2000; Strachan, 1989).

Epidemiological studies of helminth infections and autoimmune diseases raise concerns about the accuracy of this framework. Helminth infections are potent natural stimuli for TH2 responses, and they are strongly associated with TH2-type immune responses, such as high levels of IgE, eosinophilia, and mastocytosis (Yazdanbakhsh *et al*, 2001). Populations with high endemic levels of helminth infections, however, appear to be protective against atopy. Infection of mice with helminth has been shown to suppress the pulmonary allergic response to experimental allergens, such as ovalbumin (Wang *et al*, 2001). This suggests that an increase in TH2-type responses alone cannot explain the recent rise in atopic disorders. The prevalence of Type I diabetes, a TH-mediated disease, has also progressively increased in the past few decades in the same populations that have demonstrated an increase in atopic conditions (Stene and Nafstad, 2001). The increase in allergic disease and the escalation of autoimmune disorders cannot be ascribed to a simple imbalance between TH1 and TH2 responses. Failure of regulatory pathways, such as IL-10 and TGF-β, may account for these complex findings.

BACTERIAL DNA: CpG OLIGODEOXYNUCLEOTIDES

Bacterial DNA, unlike mammalian DNA, is immunostimulatory; application of bacterial DNA to mammalian immune cells leads to myriad effects, including rescue from apoptosis, induction of B cell proliferation, and stimulation of immunoglobulin secretion (Krieg *et al*, 1995). Bacterial DNA differs from mammalian DNA in two key features. First, bacterial DNA has the expected 1:16 frequency of CpG dinucleotides (cytosine and guanine with phosphodiester backbone), whereas mammalian DNA has CpG suppression, with CpG dinucleotides being found at approximately a quarter of the expected frequency (1:50–1:100) (Bird *et al*, 1987). Furthermore, when present in mammalian DNA, the majority of the cytosine in CpG dinucleotides is methylated whereas it is unmethylated in bacterial DNA. Oligodeoxynucleotides (ODN) containing DNA motifs centered around unmethylated CG dinucleotides (CpG ODN) have immune effects similar to those of native bacterial DNA. CpG ODN are probably recognized by one of the PAMP receptors. Hemmi and colleagues reported that a member of the toll-like receptor family, TLR9, recognizes bacterial DNA (Hemmi *et al*, 2000). Among the pleiotropic properties of CpG ODN is the ability to strongly induce TH1-type responses. Early studies demonstrated the induction of IL-12 (Klinman *et al*, 1996) and IFN-γ (Halpern *et al*, 1996). Thus, bacterial infections may lead to the induction of TH1 responses, at least in part through direct effects of bacterial DNA on immune cells. The postulated effects of CpG ODN include both direct and indirect effects on the commitment of CD4+ cells to a TH1 phenotype. These responses may downregulate and prevent the establishment of TH2 responses, which would diminish the manifestations of asthma and atopy. In addition, however, CpG ODN strongly induce IL-10, which inhibits both TH1 and TH2 responses in a dose-dependent manner (Kitagaki *et al*, 2002). Given the promotion of IL-10 by CpG ODN and a strong correlation between IL-10 induction and IL-5 suppression *in vitro* (Kitagaki *et al*, 2002), we speculate that the efficacy of CpG ODN in allergy may include promotion of regulatory responses as well as induction of TH1-type cytokines. Regulatory T cells (Tr1 or CD4+CD25+) have been shown to regulate TH2 responses (Suri-Payer *et al*, 1999) and downregulate antigen-specific IgE responses, promoting tolerance to allergens (Cottrez *et al*, 2000; Curotto de Lafaille *et al*, 2001). Tolerance can be transferred with CD4+CD25+ cells, which prevent the development of OVA-specific IgE, inhibit OVA-induced T lymphocyte proliferation, and suppress OVA-induced IL-4 and IL-5. Unlike the transfer of TH1 clones, this is associated with the induction of IL-10 but not IFN-γ (Cottrez *et al*, 2000). Treatment with killed *M. vaccae* confers protection against allergen-induced eosinophilic airway inflammation through the induction of CD4+CD25+ $CD45RB^{Lo}$ regulatory T cells (Zuany-Amorim *et al*, 2002). The protective effect of these cells is dependent on IL-10 and TGF-β. IL-10 is also required for the development of regulatory T cells (Akbari *et al*, 2002). These effects may be speculatively linked to CpG DNA, which may inhibit TH2-mediated responses through multiple pathways.

EFFECTS OF CpG ODN ON ALLERGIC INFLAMMATION AND MANIFESTATIONS OF ALLERGIC DISEASES

On the basis of our understanding of the effects of CpG ODN on the TH1/TH2 balance, we have examined the use of CpG ODN as a therapeutic option for allergic asthma. For these initial studies, we utilized a murine model of asthma in which C57BL/6 mice were sensitized to schistosome eggs and challenged with soluble schistosome antigen (SEA) (Kline *et al*, 1998). To determine the effect of CpG ODN in this model, we compared the development of pulmonary eosinophilia between mice that received eggs in the presence or absence of CpG ODN or control ODN. We found that mice that were pretreated with CpG ODN developed significantly fewer lung eosinophils than did those who were sensitized in the absence of ODN or in the presence of control (non-CpG) ODN. These mice were also protected against the development of a marked, multicellular peribronchial inflammatory response. We next evaluated the effect of CpG ODN on nonspecific bronchial hyperreactivity in this model by performing methacholine challenges using a whole-body plethysmograph. Mice previously sensitized to schistosome eggs and challenged with SEA developed dose-dependent methacholine-induced bronchospasm that was significantly greater than in control mice or mice pretreated with

CpG ODN, and it was no different from that in mice that received control ODN. These studies confirm that antigen-induced bronchial hyperreactivity can be prevented by CpG ODN.

In clinical asthma, eosinophilic inflammation and bronchial hyperreactivity are typically associated with TH2-type responses. In this murine model, we found that IgE induction in the inflamed mice was also reduced by treatment with CpG ODN. Moreover, elevation in BAL fluid of the TH2 cytokine IL-4 was replaced in the CpG-treated mice by elevation of IFN-γ and IL-12. Subsequent *in vitro* studies confirmed that rechallenge of splenocytes harvested from sensitized mice leads to antigen-specific release of IL-5; this induction is blocked and replaced by release of IFN-γ both when splenocytes are obtained from mice treated with CpG ODN at the time of sensitization and if the splenocytes receive CpG along with antigen *in vitro.*

Prevention of antigen-driven TH2-type responses is an important therapeutic goal. These studies indicate that if an antigen were encountered in the context of CpG DNA, subsequent exposure to the Ag in the lung would lead to a TH1 rather than a TH2 response. These data support the existence of alternate pathways, such as IL-10, in suppression of TH2 response by CpG ODN. Recent studies confirmed that the regulatory effects of IL-10 in suppressing TH2 responses are magnified in the absence of IFN-γ and IL-12 (Kitagaki *et al*, 2002). Similarities are seen in a model of allergic rhinosinusitis (Hussain *et al*, 2002).

These findings demonstrate that the attributes of a murine model of asthma characterized by IgE production, airway eosinophilia, TH2-type cytokine induction, and bronchial hyperreactivity do not develop when CpG ODN is administered at the time of allergen sensitization (Kline *et al*, 1998). As this protection is associated with induction of TH1-type responses, we next evaluated whether TH1 cytokines were necessary for the protective effects of CpG ODN. For these studies, we examined the effect of CpG ODN on the development of airway eosinophilia and bronchial hyperreactivity in the absence of IFN-γ, IL-12, or both; these experiments were carried out using both anti-cytokine-blocking antibodies and cytokine gene knockout mice (Kline *et al*, 1999). Surprisingly, we found that neither cytokine alone nor in combination was needed to permit the anti-asthma effects of CpG, although the absence of either cytokine did lead to an altered dose-response curve.

Other investigators have confirmed and extended our findings that CpG ODN are effective in abrogating asthma responses in murine models of asthma. Broide and colleagues showed that not only systemic but also mucosal administration of CpG ODN is effective; in an ovalbumin model of asthma, CpG ODN inhibited IL-5, induced IFN-γ, prevented eosinophilic inflammation (both in the lung and systemically), and decreased airway hyperresponsiveness. Their study demonstrated sustained effects, with rapid onset (within 24 hours) of inhibitory effect following a single dose of ODN (100 mcg injected i.p. or instilled intranasally). This benefit was equivalent to that following an entire week of daily corticosteroids (Broide *et al*, 1998). In another study, Sur and colleagues found that CpG ODN inhibit airway eosinophilia, IgE induction, and bronchial hyperresponsiveness using a ragweed model of murine asthma. They showed that CpG ODN were effective in preventing responses as late as six weeks following its administration (Sur *et al*, 1999). We recently demonstrated that well-established atopic responses in the lung can be reversed by immunotherapy using antigen and CpG ODN, although not by either treatment alone (Kline *et al*, 2002). Shirota and colleagues confirmed that CpG ODN are effective when given through the transmucosal route (Shirota *et al*, 2000). In addition, their findings are in agreement that concomitant administration of CpG ODN and antigen is desirable for maximal inhibitory effects (Shirota *et al*, 2000). The same group recently showed that antigen conjugated to CpG ODN is more potent than unconjugated mixture. They did not look for evidence of anti-DNA antibodies, although conjugation of DNA to proteins has been shown to enhance the likelihood of their occurrence (Shirota *et al*, 2000). Serebrisky and colleagues confirmed that CpG ODN induce TH1 cytokines (IFN-γ) and suppress TH2 cytokines (IL-4, IL-5, and IL-13) in lung lavage fluid. Uniquely, they also reported that CpG ODN decreased expression of the costimulatory molecule B7.2 and slightly increased expression of B7.1 (Serebrisky *et al*, 2000). Selective expression of B7.1 and B7.2 has been shown in many models to preferentially influence TH1 and TH2 responses, respectively (Kuchroo *et al*, 1995).

CpG appears to have similar effects on human cells as on mice, although various ODN have species specificity. Parronchi and colleagues examined the effects of CpG ODN on human antigen-specific B cells and CD4 + T cells. CpG ODN induce the *in vitro* differentiation of *Dermatophagoides pteronyssinus* (dust mite)–specific human CD4 + T cells into TH1 rather than TH2 cells. Similar to cells differentiated in the presence of exogenous IL-12, cells incubated with CpG ODN displayed diminished IL-4 production and enhanced IFN-γ production (Parronchi *et al*, 1999). These effects appeared to require the induction of IFN-γ. Fujieda and colleagues recently evaluated the effects of CpG ODN on (atopic) human peripheral mononuclear cells (PBMC) stimulated with IL-4 and anti-CD-40 monoclonal antibody. These cells developed an increase in IgE production, which was inhibited by CpG ODN. These effects are mediated by both IL-12 and IFN-γ and appear to be CpG specific (Fujieda *et al*, 2000).

CPG ODN AS AN ADJUVANT

Numerous immunological adjuvants have been described with varied potency for inducing an antibody or T cell response. Kim and colleagues compared commonly used adjuvants for two human cancer antigens (MUC1 and GD3) conjugated to an immunogenic carrier molecule, KLH (keyhole limpet hemocyanin). They measured antibody responses for IgM and IgG, T cell proliferation, and cytokine release. QS21, TiterMax, MoGM-CSF, MPL/DETOX, and CpG ODN adjuvants were effective for induction of IgM and IgG antibodies against both antigens. TiterMax and CpG ODN generated potent IFN-γ responses but less potent proliferation of IL-4 release as compared to other adjuvants (Kim *et al*, 1999). This study suggests that CpG ODN are among the most potent TH1-promoting adjuvants currently available. The use of adjuvants in immunotherapy for atopic conditions has been directed at increasing immunogenicity but not allergenicity, with the hope of reducing dose-related adverse effects. Alum-precipitated extracts have been examined for different pollens (Tari *et al*, 1997). Although these extracts have been shown to have equal safety and at least equal clinical effectiveness (Tuft, 1980), alum is known to induce antigen-specific IgE in animal models. On the other hand, Freund's complete adjuvant induces IgG production and favors a TH1 phenotype of T helper cells (Sano *et al*, 1999), but it is not appropriate for clinical use. It has been speculated that the immunoregulatory effects of killed mycobacterium bacilli might be due to the bacilli's CpG content.

CPG ODN AS A TREATMENT FOR SKIN DISORDERS

The immunoregulatory abnormalities seen in patients with atopic dermatitis—elevated serum IgE and peripheral eosinophilia—reflect increased expression of the TH2 cytokines IL-4, IL-5, and IL-13 and a concomitant decrease in IFN-γ especially during acute exacerbations (Kimura *et al*, 1998a; Kimura *et al*, 1998b). IL-10 dysregulation has been reported in different atopic conditions. Increased levels of IL-10 mRNA have been reported in asthma (Robinson *et al*, 1996) and atopic dermatitis (Ohmen *et al*, 1995), but serum levels in asthma (Borish *et al*, 1996) were low and similar to those seen as normal as compared to normal controls in atopic dermatitis (Yoshizawa *et al*, 2002). Corne and

colleagues reported reduced IL-10 levels in atopic compared with non-atopic subjects during the acute phase of upper respiratory tract infections (Corne *et al*, 2001). It has been speculated that increased local IL-10 in atopic dermatitis is secondary to constant trauma and bacterial infection. This IL-10 overexpression may be able to suppress TH1 responses but not TH2 responses, as higher levels of IL-10 are required to suppress TH2 responses in the skin (Terui *et al*, 2001).

In our mouse model of asthma, we showed that CpG ODN significantly improves hyperresponsiveness, eosinophilic inflammation, and serum IgE. They also suppress TH2 cytokines and increase TH1 cytokines. CpG ODN mainly work through TLR9 receptor on professional antigen-presenting cells and skew T cells to TH1 cells. Increased numbers of antigen-presenting cells, cutaneous dendritic cells, and Langerhans cells have been found in AD (Banfield *et al*, 2001; Leung *et al*, 1983). Jakob and colleagues showed that CpG ODN treatment of murine fetal skin–derived dendritic and Langerhans cells causes activation, mobilization, and increased production of IL-12 (Jakob *et al*, 1998). These findings were confirmed by another study that showed that intradermal or topical application of CpG ODN induces Langerhans cell migration in a manner similar to that of allergens or lipopolysaccharides (Ban *et al*, 2000). Beignon and colleagues explored the effects of CpG ODN on bare skin when combined with an antigen. They showed that the presence of CpG ODN (1826) in influenza peptide and cholera toxin preparations enhances the proliferation of peptide and virus specific T cells. They also showed that TH2 responses induced by cholera toxin are shifted to TH1, as demonstrated by an increase in IFN-γ and a decrease in IL-4, a predominance of IgG2a anti-CT antibodies in serum, and a downregulation of total serum IgE levels (Beignon *et al*, 2002). Combined data from our studies with the murine model of allergic rhinitis and limited data from skin favor the idea that CpG ODN may be an attractive therapy in the treatment of acute atopic dermatitis. On the other hand, chronic AD skin has significantly fewer IL-4 and IL-13 mRNA-expressing cells but higher numbers of IL-5, GM-CSF, IL-12, and IFN-γ mRNA expression than has acute AD skin (Leung, 1999). For that reason, the long-term benefits of treatment with CpG ODN remain speculative.

CPG ODN AND AUTOIMMUNITY

The possibility exists that CpG ODN can induce autoimmune diseases because of stimulation of TH1 responses. Data supporting this concern include the fact that CpG DNA, which is capable of activating endothelial cells, can be isolated from patients with systemic lupus erythematosus (Miyata *et al*, 2001) and the fact that activation of antigen-presenting cells by CpG ODN through TLR9 breaks immune tolerance (Ichikawa *et al*, 2002). Limited animal data have shown, however, that CpG ODN induce neither autoimmune disease nor anti-DNA antibodies in wild-type mice. Native unmodified DNA is poorly immunogenic; several studies have confirmed that double-strand DNA is an extremely poor antigen (Isenberg *et al*, 1994; Pisetsky, 1996). Although treatment of lupus-prone NZB X NZW F1 mice promotes a modest increase in the number of B cells that secrete anti-DNA IgG antibodies, there is no evidence of glomerulonephritis or autoimmune disease (Mor *et al*, 1997). On the other hand, one study found that bacterial DNA stimulates the generation of anti-DNA antibodies and increases immune-mediated glomerulonephritis in a different mouse model of SLE (Gilkeson *et al*, 1993). Other studies suggest that bacterial DNA activates autoreactive encephalitogenic T cells (Gilkeson *et al*, 1989) and induces allergic encephalomyelitis in murine models of multiple sclerosis (Segal *et al*, 2000). Finally, in primate studies wherein monkeys were treated with CpG ODN along with hepatitis B vaccine, anti-hepatitis-B titers were significantly enhanced, with no evidence of autoimmune diseases (Hartmann *et al*, 2000). Thus, although the possibility that treatment with CpG ODN may induce or promote autoimmune disorders cannot be ruled out, current evidence suggests that this is of relatively low likelihood. Of course, close attention to potential autoimmune responses must be paid in all controlled clinical trials.

FUTURE DIRECTIONS

CpG ODN are now in clinical trials for treatment of asthma and atopic disorders. Preclinical studies suggest that these agents may play an important role in the treatment of allergic diseases.

REFERENCES

Akbari O, Freeman GJ, Meyer EH, *et al*: Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity . *Nat Med* 9:1024–32, 2002

Ban E, Dupre L, Hermann E, *et al*: CpG motifs induce Langerhans cell migration in vivo. *Int Immunol* 12:737–745, 2000

Banfield CC, Callard RE, Harper JI: The role of cutaneous dendritic cells in the immunopathogenesis of atopic dermatitis. *Br J Dermatol* 144:940–946, 2001

Beignon AS, Briand JP, Muller S, Partidos CD: Immunization onto bare skin with synthetic peptides. immunomodulation with a CpG-containing oligodeoxynucleotide and effective priming of influenza virus-specific CD4+ T cells. *Immunology* 105:204–212, 2002

Bird AP, Taggart MH, Nicholls RD, Higgs DR: Non-methylated CpG-rich islands at the human alpha-globin locus. implications for evolution of the alpha-globin pseudogene. *Embo J* 6:999–1004, 1987

Bliss J, Van Cleave V, Murray K, *et al*: IL-12, as an adjuvant, promotes a T helper 1 cell, but does not suppress a T helper 2 cell recall response. *J Immunol* 156: 887–894, 1996

Borish L, Aarons A, Rumbyrt J, Cvietusa P, Negri J, Wenzel S: Interleukin-10 regulation in normal subjects and patients with asthma. *J Allergy Clin Immunol* 97:1288–1296, 1996

Broide D, Schwarze J, Tighe H, *et al*: Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J Immunol* 161:7054–706, 1998

Buffum WP, Settipane GA: Prognosis of asthma in childhood. *Am J Dis Child* 112:214–217, 1966

Corne JM, Lau L, Scott SJ, Davies R, Johnston SL, Howarth PH: The relationship between atopic status and IL-10 nasal lavage levels in the acute and persistent inflammatory response to upper respiratory tract infection. *Am J Respir Crit Care Med* 163:1101–1107, 2001

Cottrez F, Hurst SD, Coffman RL, Groux H: T regulatory cells 1 inhibit a Th2-specific response in vivo. *J Immunol* 165:4848–4853, 2000

Coyle AJ, Wagner K, Bertrand C, Tsuyuki S, Bews J, Heusser C: Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: Inhibition by a non-anaphylactogenic anti-IgE antibody. *J Exp Med* 183:1303–1310, 1996

de Curotto Lafaille MA, Muriglan S, Sunshine MJ, *et al*: Hyper immunoglobulin E response in mice with monoclonal populations of B and T lymphocytes. *J Exp Med* 194:1349–1359, 2001

Franchimont D, Galon J, Gadina M, *et al*: Inhibition of Th1 immune response by glucocorticoids: Dexamethasone selectively inhibits IL-12-induced Stat4 phosphorylation in T lymphocytes . *J Immunol* 164:1768–74, 2000

Fujieda S, Iho S, Kimura Y, Yamamoto H, Igawa H, Saito H: Synthetic oligodeoxynucleotides inhibit IgE induction in human lymphocytes. *Am J Respir Crit Care Med* 162:232–239, 2000

Gajewski TF, Goldwasser E, Fitch FW: Anti-proliferative effect of IFN-gamma in immune regulation. II. IFN-gamma inhibits the proliferation of murine bone marrow cells stimulated with IL-3, IL-4, or granulocyte-macrophage colony-stimulating factor. *J Immunol* 141:2635–2642, 1998

Gilkeson GS, Grudier JP, Karounos DG, Pisetsky DS: Induction of anti-double stranded DNA antibodies in normal mice by immunization with bacterial DNA. *J Immunol* 142:1482–1486, 1989

Gilkeson GS, Ruiz P, Howell D, Lefkowith JB, Pisetsky DS: Induction of immune-mediated glomerulonephritis in normal mice immunized with bacterial DNA. *Clin Immunol Immunopathol* 68:283–292, 1993

Halpern MD, Kurlander RJ, Pisetsky DS: Bacterial DNA induces murine interferon-gamma production by stimulation of interleukin-12 and tumor necrosis factor-alpha. *Cell Immunol* 167:72–78, 1996

Hartmann G, Weeratna RD, Ballas ZK, *et al*: Delineation of a CpG phosphorothioate oligodeoxynucleotide for activating primate immune responses in vitro and in vivo. *J Immunol* 164:1617–1624, 2000

Hemmi H, Takeuchi O, Kawai T, *et al*: A Toll-like receptor recognizes bacterial DNA. *Nature* 408:740–745, 2000

Holgate ST, Church MK: *Allergy.* London: Gower Medical Publishing, 1993

Hussain I, Jain VV, Kitagaki K, Businga TR, O'Shaughnessy P, Kline JN: Modulation of murine allergic rhinosinusitis by CpG oligodeoxynucleotides. *Laryngoscope* 112:1819–1826, 2002

Ichikawa HT, Williams LP, Segal BM: Activation of APCs through CD40 or Toll-like receptor 9 overcomes tolerance and precipitates autoimmune disease. *J Immunol* 169:2781–2787, 2002

Isenberg DA, Ehrenstein MR, Longhurst C, Kalsi JK: The origin, sequence, structure, and consequences of developing anti-DNA antibodies. A human perspective. *Arthr Rheum* 37:169–180, 1994

Jakob T, Walker PS, Krieg AM, Udey MC, Vogel JC: Activation of cutaneous dendritic cells by CpG-containing oligodeoxynucleotides: A role for dendritic cells in the augmentation of Th1 responses by immunostimulatory DNA. *J Immunol* 161:3042–3049, 1998

Kim SK, Ragupathi G, Musselli C, Choi SJ, Park YS, Livingston PO: Comparison of the effect of different immunological adjuvants on the antibody and T-cell response to immunization with MUC1-KLH and GD3-KLH conjugate cancer vaccines. *Vaccine* 18:597–603, 1999

Kimura M, Tsuruta S, Yoshida T: Correlation of house dust mite-specific lymphocyte proliferation with IL-5 production, eosinophilia, and the severity of symptoms in infants with atopic dermatitis. *J Allergy Clin Immunol* 101:84–89, 1998

Kimura M, Tsuruta S, Yoshida T: Unique profile of IL-4 and IFN-gamma production by peripheral blood mononuclear cells in infants with atopic dermatitis. *J Allergy Clin Immunol* 102:238–244, 1998

Kitagaki K, Jain VV, Businga TR, Hussain I, Kline JN: Immunomodulatory effects of CpG Oligodeoxynucleotides on Established Th2 Responses. *Clin Diagn Lab Immunol* 9:1260–1269, 2002

Kline JN, Kitagaki K, Businga TR, Jain VV: Treatment of established asthma in a murine model using CpG oligodeoxynucleotides. *Am J Physiol Lung Cell Mol Physiol* 283:L170–L179, 2002

Kline JN, Krieg AM, Waldschmidt TJ, Ballas ZK, Jain V, Businga TR: CpG oligodeoxynucleotides do not require TH1 cytokines to prevent eosinophilic airway inflammation in a murine model of asthma. *J Allergy Clin Immunol* 104:1258–1264, 1999

Kline JN, Waldschmidt TJ, Businga TR, Lemish JE, Weinstock JV, Thorne PS, Krieg AM: Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma.. *J Immunol* 160:2555–2559, 1998

Klinman DM, Yi AK, Beaucage SL, Conover J, Krieg AM: CpG motifs present in bacteria DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon gamma. *Proc Natl Acad Sci USA* 93:2879–83, 1996

Krieg AM, Yi AK, Matson S, *et al*: CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* 374:546–549, 1995

Kuchroo VK, Das MP, Brown JA, *et al*: B7–1 and B7–2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: Application to autoimmune disease therapy. *Cell* 80:707–718, 1995

Leung DY: Pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 104:S99–S108, 1999

Leung DY, Bhan AK, Schneeberger EE, Geha RS: Characterization of the mononuclear cell infiltrate in atopic dermatitis using monoclonal antibodies. *J Allergy Clin Immunol* 71:47–56, 1983

Magone MT, Chan CC, Beck L, Whitcup SM, Raz E: Systemic or mucosal administration of immunostimulatory DNA inhibits early and late phases of murine allergic conjunctivitis. *Eur J Immunol* 30:1841–1850, 2000

Martin LB, Kita H, Leiferman KM, Gleich GJ: Eosinophils in allergy. role in disease, degranulation, and cytokines. *Int Arch Allergy Immunol* 109:207–215, 1996

Matricardi PM, Bonini S: High microbial turnover rate preventing atopy. A solution to inconsistencies impinging on the Hygiene hypothesis? *Clin Exp Allergy* 30:1506–1510, 2000

Micallef MJ, Ohtsuki T, Kohno K, *et al*: Interferon-gamma-inducing factor enhances T helper 1 cytokine production by stimulated human T cells: Synergism with interleukin-12 for interferon-gamma production. *Eur J Immunol* 26:1647–1651, 1996

Miyata M, Ito O, Kobayashi H, Sasajima T, Ohira H, Suzuki S, Kasukawa R: CpG-DNA derived from sera in systemic lupus erythematosus enhances ICAM-1 expression on endothelial cells. *Ann Rheum Dis* 60:685–689, 2001

Moore KW, Vieira P, Fiorentino DF, Trounstine ML, Khan TA, Mosmann TR: Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRFI. *Science* 248:1230–1234, 1990

Mor G, Singla M, Steinberg AD, Hoffman SL, Okuda K, Klinman DM: Do DNA vaccines induce autoimmune disease? *Hum Gene Ther* 8:293–300, 1997

Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 136:2348–2357, 1986

von Mutius E, Fritzsch C, Weiland SK, Roll G, Magnussen H: Prevalence of asthma and allergic disorders among children in united Germany: A descriptive comparison. *BMJ* 305:1395–1399, 1992

von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Reitmeir P, Thiemann HH: Skin test reactivity and number of siblings. *BMJ* 308:692–695, 1994

Nakajima H, Iwamoto I, Tomoe S, Matsumura R, Tomioka H, Takatsu K, Yoshida S: CD4+ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. *Am Rev Respir Dis* 146:374–377, 1992

Ohmen JD, Hanifin JM, Nickoloff BJ, *et al*: Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed-type hypersensitivity reactions . *J Immunol* 154:1956–1963, 1995

Ohnishi T, Sur S, Collins DS, Fish JE, Gleich GJ, Peters SP: Eosinophil survival activity identified as interleukin-5 is associated with eosinophil recruitment and degranulation and lung injury twenty-four hours after segmental antigen lung challenge. *J Allergy Clin Immunol* 92:607–615, 1993

Parronchi P, Brugnolo F, Annunziato F, *et al*: Phosphorothioate oligodeoxynucleotides promote the in vitro development of human allergen-specific CD4+ T cells into Th1 effectors. *J Immunol* 163:5946–5953, 1999

Parronchi P, De Carli M, Manetti R, *et al*: IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. *J Immunol* 149:2977–83, 1992

Pisetsky DS: The immunologic properties of DNA. *J Immunol* 156:421–423, 1996

Ramirez F: Glucocorticoids induce a Th2 response in vitro. *Dev Immunol* 6: 233–243, 1998

Robinson DS, Tsicopoulos A, Meng Q, Durham S, Kay AB, Hamid Q: Increased interleukin-10 messenger RNA expression in atopic allergy and asthma. *Am J Respir Cell Mol Biol* 14:113–117, 1996

Sano K, Haneda K, Tamura G, Shirato K: Ovalbumin (OVA) and Mycobacterium tuberculosis bacilli cooperatively polarize anti-OVA T-helper (Th) cells toward a Th1-dominant phenotype and ameliorate murine tracheal eosinophilia. *Am J Respir Cell Mol Biol* 20:1260–1267, 1999

Santeliz JV, Van Nest G, Traquina P, Larsen E, Wills-Karp M: Amb a 1-linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma. *J Allergy Clin Immunol* 109:455–462, 2002

Sears MR: International trends in asthma mortality. *Allergy Proc The* 12:155–158, 1991

Segal BM, Chang JT, Shevach EM: CpG oligonucleotides are potent adjuvants for the activation of autoreactive encephalitogenic T cells in vivo. *J Immunol* 164:5683–5688, 2000

Serebrisky D, Teper AA, Huang CK, *et al*: CpG Oligodeoxynucleotides can reverse Th2-associated allergic airway responses and alter the B7.1/B7.2 expression in a murine model of asthma. *J Immunol* 165:5906–5912, 2000

Shaheen SO, Aaby P, Hall AJ, Barker DJ, Heyes CB, Shiell AW, Goudiaby A: Measles and atopy in Guinea-Bissau. *Lancet* 347:1792–1796, 1996

Shirakawa T, Enomoto T, Shimazu S, Hopkin JM: The inverse association between tuberculin responses and atopic disorder. *Science* 275:77–79, 1997

Shirota H, Sano K, Kikuchi T, Tamura G, Shirato K: Regulation of murine airway eosinophilia and Th2 cells by antigen-conjugated CpG oligodeoxynucleotides as a novel antigen-specific immunomodulator. *J Immunol* 164:5575–5582, 2000

Shirota H, Sano K, Kikuchi T, Tamura G, Shirato K: Regulation of T-helper type 2 cell and airway eosinophilia by transmucosal coadministration of antigen and oligodeoxynucleotides containing CpG motifs. *Am J Respir Cell Mol Biol* 22:176–182, 2000

Sinigaglia F, D'Ambrosio D, Rogge L: Type I interferons and the Th1/Th2 paradigm. *Dev Comp Immunol* 23:657–663, 1999

Stene LC, Nafstad P: Relation between occurrence of type 1 diabetes and asthma. *Lancet* 357:607–608, 2001

Strachan DP: Hay fever, hygiene, and household size. *BMJ* 299:1259–1260, 1989

Sur S, Wild JS, Choudhury BK, Sur N, Alam R, Klinman DM: Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligodeoxynucleotides. *J Immunol* 162:6284–6293, 1999

Suri-Payer E, Amar AZ, McHugh R, Natarajan K, Margulies DH, Shevach EM: Post-thymectomy autoimmune gastritis. Fine specificity and pathogenicity of anti-H/K ATPase-reactive T cells. *Eur J Immunol* 29:669–677, 1999

Swain SL, Weinberg AD, English M, Huston G: IL-4 directs the development of Th2-like helper effectors. *J Immunol* 145:3796–3806, 1990

Tari MG, Mancino M, Ghezzi E, Frank E, Cromwell O: Immunotherapy with an alum-adsorbed Parietaria-pollen allergoid: a 2- year, double-blind, placebo-controlled study. *Allergy* 52:65–74, 1997

Terui T, Sano K, Shirota H, *et al*: TGF-beta-producing CD4+ mediastinal lymph node cells obtained from mice tracheally tolerized to ovalbumin (OVA) suppress both Th1- and Th2- induced cutaneous inflammatory responses to OVA by different mechanisms. *J Immunol* 167:3661–3667, 2001

Tighe H, Takabayashi K, Schwartz D, *et al*: Conjugation of immunostimulatory DNA to the short ragweed allergen amb a 1 enhances its immunogenicity and reduces its allergenicity. *J Allergy Clin Immunol* 106:124–134, 2000

Tuft L: Treatment of hay fever with alum precipitated pyridine (Allpyral) ragweed pollen extracts: A clinical reappraisal. *Ann Allergy* 44:279–282, 1980

Wang CC, Nolan TJ, Schad GA, Abraham D: Infection of mice with the helminth Strongyloides stercoralis suppresses pulmonary allergic responses to ovalbumin. *Clin Exp Allergy* 31:495–503, 2001

Yazdanbakhsh M, van den Biggelaar A, Maizels RM: Th2 responses without atopy. Immunoregulation in chronic helminth infections and reduced allergic disease. *Trends Immunol* 22:372–377, 2001

Yoshizawa Y, Nomaguchi H, Izaki S, Kitamura K: Serum cytokine levels in atopic dermatitis. *Clin Exp Dermatol* 27:225–229, 2002

Zuany-Amorim C, Sawicka E, Manlius C, *et al*: Suppression of airway eosinophilia by killed Mycobacterium vaccae- induced allergen-specific regulatory T-cells. *Nat Med* 8:625–629, 2002