

Allergy Review Series VII: Intracellular signaling and regulation of allergic reactions

CpG oligonucleotide modulation of allergic inflammation

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Mechanisms of allergy and asthma

The increase in our knowledge of the cellular and molecular mechanisms of allergic disease has been accompanied by rising trends in the incidence and severity of these diseases worldwide, and an ever increasing need for effective treatments. Currently, almost half of the population of the Western nations demonstrates sensitization to one or more environmental allergens (1). Asthma, one of the most serious allergic diseases in terms of impact on health, has become an epidemic in many parts of the world, with the highest prevalence of up to 29% in Western countries (1). Epidemiologic studies indicate a global increase in morbidity and mortality rate from asthma despite an increasing arsenal of therapeutic agents.

Allergic inflammation is thought to be driven by exposure to allergens through IgE-dependent mechanisms, resulting in an inflammatory response characterized by tissue infiltration by eosinophils. During seasonal allergen exposure, the ragweed-specific IgE level increases to the extent that it can account for 50% of total serum IgE (2). Allergen-specific IgE plays an important role in eosinophil recruitment during the allergic late-phase inflammatory response (3, 4).

Eosinophils in turn play an important role in the pathogenesis of asthma and allergic inflammation by mediating injury to the mucosal surfaces including bronchial mucosa (5). Eosinophil granule proteins increase airway reactivity to acetylcholine, and thus may contribute to the bronchoconstriction characteristic of asthma (5–7).

In addition to B cells producing IgE and infiltrating eosinophils, many other inflammatory cells are involved in allergic inflammation including mast cells, basophils, macrophages, dendritic cells, neutrophils, natural killer (NK) cells, epithelial cells, and T cells. These cells produce a host of inflammatory mediators, including histamine, cysteinyl leukotrienes, chemokines, and cytokines, which are involved in initiating, regulating, and amplifying the allergic inflammatory response (8).

Increased understanding of the pathogenesis of allergy and asthma has resulted from the discovery that T cells can produce a specific array of proinflammatory cytokines in response to allergens (9). These Th2 cytokines produced by a subset of T helper cells include interleukin (IL)-4, IL-5, IL-6, IL-9, IL-10, and IL-13 (10, 11). An increasing body of literature indicates the significant role played by Th2 cytokines

in mediating allergic indices such as eosinophilic allergic inflammation, bronchial hyperresponsiveness (BHR), and the formation of allergen-specific IgE (10, 12–15). T helper cells are not the only source of Th2 cytokines. CD8⁺ T cells, mast cells, basophils, and eosinophils can also produce Th2 cytokines, and in certain instances these cellular sources may play important roles in the allergic response (8). IL-4, the cytokine primarily responsible for stimulating the differentiation of Th2 cells from naive T helper cells, is secreted by several non-T helper cell types including mast cells and NK T cells. The early production of IL-4 from these cellular sources may trigger Th2 cell differentiation, and initiate the progressive formation of a dominant allergic phenotype.

In contrast, another T helper cell subtype, Th1 cells, produce interferon (IFN)- γ which tends to oppose the production and actions of IL-4, and inhibit allergic responses. The differentiation of Th1 cells from naive T helper cells is primarily induced by IL-12, which is produced by macrophages and dendritic cells. We and others have shown that IL-12 and IFN- γ inhibit allergen-induced eosinophil recruitment, Th2 cytokine synthesis, airway hyperresponsiveness, and IgE synthesis in murine models of asthma (16–20). The results of these studies suggest that agents that promote a Th1 phenotypic bias by promoting the production of IL-12 and IFN- γ and/or restricting the production of Th2 cytokines may have the potential to limit human allergic disease including asthma.

CpG DNA: epidemiologic evidence for a protective effect

The possibility that CpG DNA of bacterial or viral origin may protect against allergic disease is supported by several epidemiologic studies. In a study examining the relationship between measles and atopy, children that had been infected with measles, in contrast to vaccination, demonstrated a significantly reduced risk of atopy (21). Similarly, a recent study by Shirakawa et al., reported that a positive tuberculin response predicted a lower incidence of asthma and atopy in Japanese schoolchildren, and the tuberculin responsiveness correlated with increased Th1 and reduced Th2 serum cytokines (22). The results of two studies demonstrating an inverse relationship between the number of siblings and atopy suggest that the early onset of childhood illnesses transmitted by older siblings may also protect against the later development of atopy (23, 24). Similarly, a recent study involving over 1000 children found that exposure of young children to other children at day-care facilities or to older siblings at home protected against the development of asthma and frequent wheezing later in childhood (25). These results suggest that bacterial or viral infections may confer long-lasting immune effects,

perhaps via immune deviation from a Th2 to Th1 bias, with a concomitant reduction in the risk of atopy and asthma. It remains to determine whether CpG DNA motifs are the primary modulators of this effect, or whether other factors, such as bacterial cell-wall components, play a role.

History

The first reports of the immunostimulatory properties of bacterial DNA appeared in the late 1980s. Tokunaga and others found that DNA obtained from the *Bacillus Calmette-Guérin* (BCG) produced antitumor activity via activation of NK cells (26–28). Subsequently DNA from bacteria, but not from vertebrates, was shown to activate NK cells and stimulate IFN- γ secretion (29), and a 45-mer oligodeoxynucleotide (ODN) was identified containing a palindromic sequence motif with a central C-G dinucleotide which was essential for the activating effects (26, 27, 30). At about the same time, in conjunction with antisense research, several researchers noted that certain ODNs induced proliferation of B cells. Krieg, Klinman, and others defined the motif underlying these effects on B cells as short ODNs containing a central unmethylated C-G dinucleotide (termed CpG) flanked by two 5' purines and two 3' pyrimidines (31). This motif was 20 times more abundant in bacterial DNA than in vertebrate DNA, and was almost identical to the 45-mer ODN derived from BCG DNA that activated NK cells and stimulated IFN- γ production (32).

Defining CpG ODN activity

Despite the early sequence characterizations, the precise DNA sequence structure required for CpG ODN-induced immune stimulation is only partially understood (33). Two aspects of the stimulatory DNA structure which do seem to be generally required are the inclusion of adjacent cytosine and guanine residues (CpG) in the ODN, and the lack of C-5 methylation of the cytosine at the CpG core (31, 34–37). Unmethylated CpG dinucleotides are abundant in all bacterial genomes (38), as well as some viral (39, 40) and invertebrate eukaryotic genomes (41). In vertebrate genomes, CpG motifs can be found only at low frequency, and about 70% of vertebrate CpG DNA is methylated (38, 42).

Mechanisms of action

The character of CpG DNA receptors is controversial. One study demonstrated that Sepharose beads coated with CpG DNA stimulated B cells as effectively as free CpG DNA, indicating the presence of a cell surface-receptor. However, DNA is actively taken up by all

types of cells, and other studies have reported that CpG ODN uptake via active endocytosis is an essential step in the manifestation of CpG ODN specific effects (43–45). The early mechanistic events underlying the immune stimulatory effects of CpG ODN following endocytosis are not well characterized; however, some steps are known. Most of the DNA remains in the endosomes, but a small fraction appears to reach the nucleus (45–47). Acidification of the endosomes appears to be essential to the actions of CpG ODN, as drugs which block endosomal acidification block immune activation by CpG ODN (48). However, the precise mechanism by which endosomal acidification confers activity on CpG ODN is not known.

The intracellular protein receptors for CpG DNA have not been fully elucidated. Cytoplasmic and nuclear factors which bind unmethylated CpG motifs reportedly have been detected, and efforts to purify the binding proteins are currently underway (49). Moreover, two proteins which may bind CpG DNA have recently been shown to mediate CpG DNA-induced effects. DNA-dependent protein kinase (DNA-PK) was reported to be required for the activation of innate immunity by immunostimulatory DNA (50). In this study, administration of bacterial DNA or CpG ODN to mice lacking the catalytic subunit of DNA-PK, and *in vitro* CpG stimulation of bone-marrow-derived macrophages from these mice, inhibited activation of NF- κ B and reduced induction of IL-6 and IL-12. DNA-PK are present in both the cytoplasm and nucleus, and the site-specific interaction of CpG DNA with DNA-PK, as well as associated signaling pathways other than NF- κ B activation, remain to be characterized. Another protein, Toll-like receptor 9 (TLR9), was also recently shown to mediate CpG ODN-induced splenocyte proliferation, dendritic cell maturation, and macrophage production of tumor necrosis factor (TNF)- α , IL-6, and IL-12 (51). This study also demonstrated that CpG ODN-induced *c-Jun* NH₂-terminal kinase (JNK) and IL-1 receptor kinase (IRAK) activation were reduced in TLR9-deficient mice, and that these mice were resistant to CpG ODN-augmented toxic shock. The cellular location of TLR9 has not been described yet. However, the presence of a transmembrane segment in the TLR9 gene strongly suggests that it is a membrane protein, and this is consistent with a possible location on endosomal membranes.

Various signaling molecules have been identified that participate in signaling pathways upstream of CpG ODN-induced cytokine production. A common Toll receptor signaling pathway includes the adapter protein, myeloid differentiation marker 88 (MyD88), and this molecule has been shown to mediate CpG ODN-induced IL-12 expression and TNF- α production (52, 53). MyD88 in turn is thought to interact with IRAK molecules, followed by interaction with TNF receptor-associated factor (TRAF)6. TRAF6 has also been

shown to mediate CpG ODN-induced IL-12 production in a murine macrophage cell line (53). The mitogen-activated protein kinase (MAPK) p38 and JNK pathways are downstream from TRAF6 and have been shown to be stimulated by CpG ODN in B cells and macrophages. Inhibition of p38 MAPK with the specific inhibitor, SB203580, inhibits CpG-ODN-induced IL-12 production by B cells and macrophages, indicating that this pathway mediates the production of at least one of the CpG-induced cytokines (54–56). Specific inhibitors of p38 have also been shown to inhibit allergic responses (57), and as CpG ODN have also been shown to block allergic responses, this paradoxical effect serves to emphasize that the mechanisms underlying the actions of CpG ODN are complex, with many interactive factors and pathways remaining to be elucidated. In addition to activation of the transcription factor, NF- κ B (58), CpG ODN also activate other transcription factors including *c-myc*, and Ets-2 (59, 60). CpG ODN have been reported to stimulate rapid intracellular production of reactive oxygen species (ROS), which mediate CpG ODN-induced IL-6 expression (61, 62), but the enzymatic source and types of ROS have not been identified. The putative CpG DNA signaling pathways are shown in Fig. 1.

Downstream of the signaling pathways, CpG DNA has been shown to induce gene expression of various cytokines including IL-12, IL-6, IL-1 β , IL-18, TNF- α , and IFN- α , - β , and - γ , and chemokines including MIP-1 β and MCP-1 in specific subsets of innate and acquired immune cells (28, 58, 63–68). The actions of CpG DNA on antigen-presenting cells (APC), B cells, NK cells, and T cells are described below, and the cellular sources and targets of CpG DNA-induced cytokines are shown in Fig. 2.

CpG ODN modulation of macrophage and dendritic cell function

CpG DNA directly activates two important classes of APC, macrophages and dendritic cells. Macrophages activated by CpG ODN produce the Th1-inducing cytokines IL-12 and IL-18, as well as TNF- α and IFN- α/β (37, 58, 63, 69, 70). These cytokines potently stimulate the production of IFN- γ from NK cells and Th1 cells. Since IFN- γ activates macrophages further and stimulates macrophage production of IL-12 and IL-18, a positive feedback loop is formed (71). In addition, the antigen-processing and presentation apparatus of macrophages is also amplified by CpG ODN stimulation. Bone-marrow-derived macrophages upregulate expression of MHC class I, CD40, intracellular adhesion molecule 1, and CD16/32 in response to CpG ODN (72). Like mouse macrophages, human macrophages are activated by CpG ODN (70).

Dendritic cells are the major APC in the lung, and are

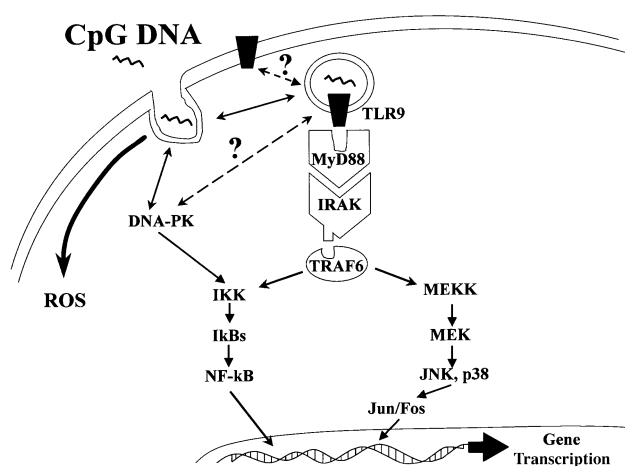


Figure 1. CpG DNA signaling pathways. This diagram summarizes the intracellular molecules and pathways that are thought to mediate CpG DNA signal transduction. A clear understanding of the actions and interactions of many of the signaling molecules remains incomplete, including those of CpG DNA-stimulated ROS. Also unknown is the cellular location of TLR9; as indicated by the question mark, it may occur on the plasma membrane or on an endosomal membrane. Similarly, it is not known whether DNA-PK directly or indirectly influences the TLR9 signaling pathway or whether TLR9 influences the DNA-PK signaling pathway.

thought to play a primary role in priming the immune response (73). As with macrophages, CpG ODN stimulate dendritic cells to produce IL-6, IL-12, and TNF- α , as well as increase surface expression of MHC class II, CD40, CD54, and CD86 molecules (63, 74, 75). In addition, CpG ODN-treated dendritic cells demonstrated enhanced functional activity in mixed lymphocyte reactions (74, 75), and induced T cells to secrete increased levels of Th1 cytokines (74). Human primary dendritic precursor cells and peripheral blood dendritic cells, but not monocyte-derived dendritic cells, were shown to be activated by CpG ODN (74).

CpG ODN modulation of B-cell function

B-cell modulation resulting in restricted IgE production or the production of Th1 cytokines has important implications for allergy. CpG ODN have been reported to stimulate murine B cells to produce IL-6 and IL-10 (61, 76) as well as IL-12 (77). CpG ODN induction of IL-12 may conceivably influence Th1 differentiation; however, the B-cell contribution relative to dendritic cell or macrophage IL-12 production is not known (77, 78). The CpG ODN-induced production of IL-6 from B cells is augmented by IFN- γ (79), suggesting that B-cell production of IL-12 may be amplified by the combination of IFN- γ and CpG ODN. CpG ODN also amplify B-cell function and survival in other ways, including direct and potent stimulation of B-cell mitogenesis. CpG ODN stimulate both resting and activated B cells,

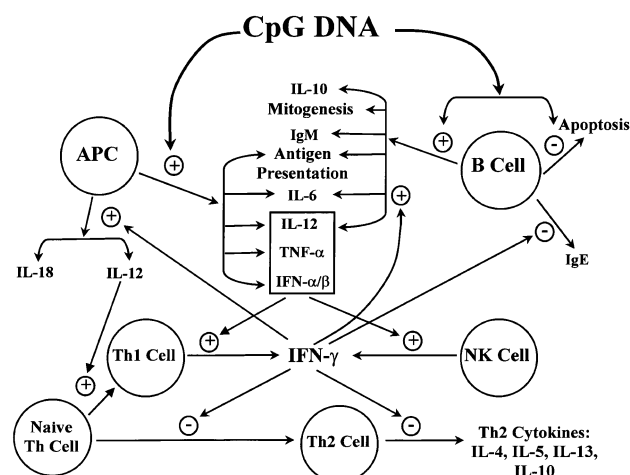


Figure 2. Cellular sources and targets of CpG DNA-induced cytokines. This diagram summarizes cellular mechanisms of CpG DNA stimulation, including cytokine products and selected cytokine actions. Plus (+) symbols indicate positive stimulation; minus symbols (–) indicate inhibition.

and are capable of driving more than 95% of B cells into the cell cycle (31). In addition, CpG ODN stimulate increased expression of MHC class I and II molecules and B7-1 and B7-2 costimulatory molecules on B cells (72), and rescue B cells from apoptosis in experimental models (80–82), suggesting that CpG ODN may enhance antigen presentation by B cells and B-cell survival. In terms of immunoglobulin secretion, CpG ODN-activated B cells are induced to secrete IgM in an IL-6-dependent fashion (80). CpG ODN reportedly downregulate the low-affinity IgE receptor, CD23 (72), but CpG ODN have not been reported to directly modulate IgE immunoglobulin class switching.

Human B cells reportedly respond to CpG ODN in a manner similar to murine B cells, although the optimal ODN motifs for stimulation are different (83, 84).

CpG ODN modulation of NK cell function

NK cells have been reported to contribute to the development of antigen-specific IgE, increased bronchoalveolar lavage (BAL) levels of IL-4 and IL-5, and eosinophilic airway inflammation in a murine model of allergic asthma (85). Early studies demonstrated that CpG ODN potently stimulates NK lytic activity and IFN- γ production. Both mouse and human blood NK cells are responsive to CpG ODN (34). The significance of this effect is underscored by the observation that more than 90% of the early IFN- γ -producing cells are NK cells (86). Purified NK cells are not directly stimulated by CpG ODN, but require IL-12, TNF- α , and IFN- α/β from APC. Thus, most of the effects of CpG-ODN on NK cells seem to be indirect, but NK cells are also

directly costimulated by CpG ODN and IL-12, as this combination more strongly stimulated NK cells than IL-12 alone (86).

CpG ODN modulation of T-cell function

The effects of CpG ODN on T cells also appear to be indirect. Treatment of murine splenocytes with anti-CD3-activating antibody combined with CpG ODN resulted in increased levels of CD69 and B7-2 on T cells and increased production of IFN- γ (41, 87). This effect was mediated by type I IFN and IL-12 produced by APC. The effect of CpG ODN on T cells should not be discounted with regard to the protective effects of CpG ODN against allergic inflammation, because memory CD4⁺ and CD8⁺ T cells mediate long-term Th1 phenotypic responses to antigens (88–90).

The protective effects of CpG ODN in mouse models of asthma

Several laboratories including our own have shown that CpG ODN can be used to prevent or treat allergic disease in rodent models (91–97). In an initial study employing a murine model of asthma and schistosome egg allergen, Kline et al., demonstrated that CpG ODN reduced BAL eosinophilia, BHR, serum IgE, and BAL IL-4 levels, while increasing BAL IFN- γ and IL-12 levels (91). CpG ODN provided protective effects when administered in conjunction with the sensitizing dose of allergen, or when administered after an initial allergic sensitization. In another study, CpG ODN were shown to reduce eosinophilia in BAL, lung parenchyma, serum, and bone marrow, suggesting that CpG ODN inhibited lung eosinophil accumulation, and also progenitor differentiation of eosinophils (98). In the same study, both intraperitoneally and mucosally administered CpG ODN were shown to be effective; the inhibition of eosinophilia was associated with decreased generation of IL-5, GM-CSF, and IL-3, but not increased eosinophil apoptosis; and the effects were partially mediated by IL-12 and types I and II IFN. In a study performed in our laboratory, CpG ODN inhibited allergic lung inflammation 6 weeks after the last administration of CpG ODN in a long-term model murine model of allergic asthma (92). The long-term protective effects of CpG ODN were shown to be associated with decreased serum IgE, an increased ratio of IFN- γ - to IL-4-secreting cells, and increased antigen-specific production of IFN- γ by spleen cells.

Immunotherapy

The main limitations of allergen immunotherapy as currently practiced are that it is a time-consuming, inconvenient procedure, and entails a risk of anaphy-

laxis. In the past, allergists used bacterial vaccines of heat-killed organisms as adjuvants with allergen immunotherapy, but the results were equivocal (99). Two recent studies using CpG ODN coupled with allergen-encoding plasmid DNA or coupled directly to allergen indicate that the efficacy and safety of specific immunotherapy can be improved with CpG ODN.

In the first study, Balb/c mice injected intradermally with plasmid-encoding Bet v 1, the major allergen of birch pollen, in conjunction with CpG ODN demonstrated reduced humoral responses against Bet v 1, and stimulated greater production of IFN- γ from allergen-stimulated spleen cells than mice injected with the Bet v 1-encoding plasmid alone (100). This study demonstrated that CpG ODN could increase the ability of intradermal injections of allergen DNA to stimulate a Th1 phenotype, but did not determine whether this therapeutic strategy could interrupt or downregulate an ongoing Th2 response, as would be necessary for specific immunotherapy of an established allergic disease.

In the second study, CpG ODN coupled to the short ragweed allergen, Amb a 1, was used to determine whether intradermal injections could stimulate a primary Th1 immune response, prevent a subsequent Th2 response, or interrupt a pre-established Th2 response (101). A single intradermal injection of the CpG ODN-Amb a 1 conjugate in BALB/c mice was shown to stimulate production of serum IgG2a, and increase production of IFN- γ from Amb a 1-activated spleen cells derived from treated mice 16 weeks after treatment, indicating that the conjugate stimulated a strong and prolonged Th1 response. These results were not produced by injections of Amb a 1 alone or Amb a 1 conjugated to a non-CpG ODN. The CpG ODN-allergen conjugate was also shown to stimulate serum IgG2a and IFN- γ production from Amb a 1-activated splenocytes as well as reduce serum IgE in mice boosted with a Th2-stimulating dose of intraperitoneal Amb a 1 plus alum, or in mice initially converted to a Th2 phenotype with intraperitoneal Amb a 1 plus alum. Thus, in conditions which mimicked the clinical situation of a patient receiving allergen immunotherapy, a conjugate of CpG ODN and ragweed allergen was shown to produce prolonged efficacious results. The CpG ODN-allergen conjugate also demonstrated increased immunogenicity compared to Amb a 1 alone in rabbits and cynomolgus monkeys, as represented by the induction of a high titer of allergen-specific serum IgG antibodies. Less allergenicity, however, was produced by the CpG ODN-Amb a 1 conjugate than Amb a 1 alone, as the conjugate stimulated a 30-fold lower histamine release from basophils obtained from patients with ragweed allergy. These results indicate that future immunotherapy with CpG ODN-allergen

conjugates may provide enhanced efficacy while minimizing allergenicity and the potential for anaphylactic reactions.

Other allergic diseases

CpG ODN have also recently been shown to limit effectively the early- and late-phase inflammation in a murine conjunctivitis model (102). In this study, systemic or mucosal administration of CpG ODN after allergic sensitization inhibited the immediate hypersensitivity response, late-phase IgE response, and cellular infiltration. These results suggest that CpG ODN may be useful for the treatment of allergic diseases not centered in the lung.

The importance of IL-12 and IFN- γ in mediating the protective effects of CpG ODN

Several studies have demonstrated that CpG ODN stimulate APC to produce the Th1-inducing cytokine, IL-12, which in turn stimulates the production of IFN- γ from NK and T cells. Because both IL-12 and IFN- γ potentially inhibit allergic inflammation in animal models of asthma, it is a reasonable hypothesis that one or both of these cytokines may mediate the anti-inflammatory effects of CpG ODN. Both IFN- γ (92) and IL-12 (98, 102) have been reported to mediate some of the protective effects of CpG ODN in allergic models. A more definitive study determined that neither of these cytokines was an essential mediator of the CpG ODN-antiinflammatory effects in a murine model of allergic asthma (103). This study, which utilized IFN- γ IL-12 KO mice and IFN- γ /IL-12 double-KO mice, demonstrated that CpG ODN could reduce both airway eosinophilia and BHR in the absence of IFN- γ and/or IL-12, but a larger dose of CpG ODN was needed than when either of the cytokines was present. Thus, it appears that, at least, in one murine model, IL-12 and IFN- γ were involved in the anti-inflammatory effects of CpG ODN, but other factors were also critical mediators. A recent study examining CpG ODN inhibition of schistosome egg-induced disorder demonstrated that the CpG ODN inhibition of Th2 responses involved IL-12-independent upregulation of IL-10, and the costimulatory factors B7.1 and CD40 (104). Another CpG ODN-induced factor, IL-18, has been shown to reduce airway eosinophilia under specific conditions, but has not been assessed for its role in mediating the anti-inflammatory effects of CpG ODN. However, IL-18 would seem to be a poor candidate, however, as indicated by recent reports that it can stimulate increased airway eosinophilia, serum IgE, Th2 cytokine production, and allergic sensitization in murine models of allergic asthma (105–107).

Human data

CpG ODN have also been shown to convert human cell populations derived from atopic individuals from a Th2 bias to a Th1 bias. In peripheral blood mononuclear cells (PBMC) obtained from atopic patients, CpG ODN stimulated a significant increase in IL-12 and IL-18 mRNA and IFN- γ protein (108). In addition, CpG ODN decreased total, but not allergen-specific, IgE. In another study, specific ODNs induced B-cell proliferation and shifted the *in vitro* differentiation of T cells obtained from atopic donors from a Th2 profile to a Th1 profile (109). The latter effect was blocked by neutralizing antibodies to IL-12, IFN- γ , and IFN- α . While cytosine methylation abolished the Th1-inducing activity of ODNs, CpG motifs were not required in the active ODN. Similarly, CpG ODN were shown to inhibit the production of IgE from PBMC stimulated with IL-4 and anti-CD40 *in vitro*, and the inhibition was mediated by IFN- γ and IL-12 (110). These results underscore the hypothesis that CpG ODN and other ODNs can produce similar effects in human cells and in mouse models of allergy; however, the active ODN sequences are different.

The data derived from mouse allergy models and human *in vitro* experiments suggest that CpG ODN may be efficacious for the treatment of allergic diseases, or as an adjuvant in immunotherapy. In an initial phase 1 safety study, a conjugate of CpG ODN and the ragweed allergen, Amb a 1, was shown to be less allergenic in puncture skin testing than ragweed extract (111). Currently, clinical trials examining the combination of CpG ODN and allergen for the treatment of allergic rhinitis are underway.

Potential toxicity of CpG ODN

The possibility exists that CpG ODN administration may produce acute toxicity associated with their immune stimulatory effects. Schwartz et al. reported that intratracheal instillation of CpG ODN increased production of TNF- α and IL-6, and produced a neutrophilic respiratory tract inflammation 4 h after instillation (65). However, the same researchers reported that CpG ODN inhibited LPS-induced neutrophilic lung inflammation, suggesting that CpG ODN can mediate both acute pro- and anti-inflammatory effects (112). Because IL-6 and TNF- α exacerbate septic shock, CpG ODN might augment septic shock in the presence of other agents which stimulate septic shock such as lipopolysaccharide (LPS). CpG ODN administered in combination with sublethal doses of LPS has been shown to increase lethal septic shock in mice (86). Similarly, TNF- α production by macrophages stimulated with CpG ODN has been shown to mediate lethal shock in D-galactosamine-sensitized mice (113). While these studies indicate the possibility that

CpG ODN may exacerbate septic shock initiated by other factors, CpG ODN alone has not been reported to produce septic shock.

Several autoimmune diseases are Th1 in nature, and there is concern that administration of CpG ODN may precipitate or exacerbate autoimmune reactions in susceptible individuals. Studies with mice have reported that bacterial DNA induced allergic encephalomyelitis (114), and stimulated the generation of anti-DNA antibodies, and immune-mediated glomerulonephritis in a model of systemic lupus erythematosus (115). Recently, intra-articular injection of CpG ODN has been shown to induce arthritis in a mouse model of septic arthritis; however, antigen-specific autoimmunity was not demonstrated (116). In man, CpG ODN stimulated IFN- α , which is increased in the blood of patients with systemic lupus erythematosus (SLE), and correlates with disease activity (117).

Bacterial infection is suspected to be a triggering factor for SLE. As noted previously, CpG ODN have been shown to induce polyclonal B-cell activation, overexpression of immunostimulatory cytokines, and resistance to apoptosis. These actions could combine to promote the production and survival of autoreactive cells, and thus contribute to autoimmune disease. Patients with SLE have also been reported to have elevated levels of circulating plasma DNA, and genomic DNA that is enriched with hypomethylated CpG motifs (118–120). Interestingly, chloroquine and quinacrine, drugs used for the treatment of SLE and rheumatoid arthritis, have been shown to inhibit the antiapoptotic and cytokine-inducing actions of CpG ODN (48). Taken together, these studies suggest a possible pathologic role for CpG ODN in SLE.

Therapeutic strategies

Conceivably, the toxicity of CpG ODN can be minimized and the therapeutic actions maximized by

optimizing the packaging and route of administration of the CpG ODN (Table 1). Two studies have reported that CpG ODN conjugated to allergen peptides increased the potency and immunogenicity of the allergen peptides relative to administration of the mixtures of CpG and peptide or peptide alone (93,101). Similarly, plasmid DNA vaccines containing allergen-peptide encoding inserts and CpG motifs have been shown to be more immunogenic than plasmid DNA vaccines lacking CpG motifs (100).

These two methods of allergen peptide administration minimize the amount of peptide administered, and thus reduce the possibility of anaphylaxis. CpG motifs, by stimulating a Th1 immune bias, also limit the production of IgE, and reduce the danger of anaphylaxis in this manner. In addition, as mentioned previously, the conjugation of CpG to the ragweed antigen, Amb a 1, has been shown to reduce the affinity of IgE binding to Amb a 1 (101).

The CpG ODN–allergen peptide conjugate in particular has the potential to stimulate the differentiation of allergen-specific Th1 cells as opposed to producing a global Th1 bias. After this treatment, allergen-specific Th1 cells would be expected to reduce allergic responses to a particular antigen, but other antigens might still be expected to elicit a Th2 response. By stimulating only specific Th1 responses, the potential risk of autoimmune disease associated with a global Th1 bias may be reduced. This strategy, however, would be more difficult to pursue in individuals with sensitivity to multiple allergens, as is usually the case. In this situation, eliciting Th1 responses to multiple allergens might be therapeutically desirable, but the risk of a global Th1 bias is consequently increased.

Another method of limiting the systemic effects of CpG ODN may be to limit its distribution by targeting its administration to mucosal surfaces in organs such as the lung. The lung is a particularly appropriate target because many aeroallergens are first encountered in air

Table 1. Potential strategies for clinical application of CpG DNA

	Advantages	Disadvantages
Packaging		
1. CpG alone	• Effective against multiple allergens	• May produce global Th1 response, and potentiate autoimmune disease
2. CpG–allergen peptide conjugates	• Reduced anaphylaxis to allergen	• Difficult to adapt to multiple allergens
3. Plasmid DNA encoding allergen peptides and CpG motifs	• Allergen-specific Th1 differentiation	
	• Reduced anaphylaxis to allergen	• Difficult to adapt to multiple allergens
	• Allergen-specific Th1 differentiation	
Routes of administration		
1. Intrapulmonary	• Potentially limited distribution and reduced systemic toxicity	• Reduced distribution of desirable effects
2. Systemic	• Broader distribution of desirable effects	• Potentially greater systemic toxicity
Schedules of administration		
1. Prevention of allergy	• Possible long-term immune deviation	• Greater potential for induction of autoimmune disease
		• Difficult to predict sensitizing allergens
2. Treatment of existing allergy	• Can prescreen for sensitizing allergens	• Possibly difficult to deviate established immune responses

breathed into the lung. Thus, it seems logical to attempt to stimulate a local Th1 immune response to process aeroallergens safely, and at the same time limit the distribution of agents, such as CpG ODN, which initiate the Th1 response. This strategy has been successfully implemented in animal studies with a cytokine product of CpG ODN, IL-12. Intratracheal administration of IL-12 into the lung has been shown to reduce dramatically the amount of systemically circulating IL-12, compared to intraperitoneal administration of IL-12 (121). The lung administration of IL-12 has also been shown to reduce allergen-induced airway eosinophilia and airway hyperresponsiveness more potently than systemically administered IL-12, thus allowing lower therapeutic doses (121, 122). In another study, the administration of a low dose of IL-12 (50 µg) to the lung produced less systemic toxicity than a higher dose (500 µg) (123). These data suggest that the therapeutic index of IL-12 can be increased for the treatment of allergic asthma by targeted administration to the lung, and a similar effect may be achievable by targeted administration of CpG ODN to the lung. Intratracheally administered CpG ODN are rapidly taken up by alveolar macrophages, followed by increased macrophage expression of IL-12 p40 mRNA in 2 h (124). Since alveolar macrophages are localized to the lung, the systemic distribution of CpG ODN may be further restricted in this manner.

Concluding remarks

A recent suggestion has been that an asthma vaccine might be achievable (125). This vaccine would be

designed to induce protective Th1 responses to prevent allergic sensitization and the development of atopic disease. Because CpG ODN are potent inducers of Th1-inducing cytokines, these agents would seem to be ideal candidates for inclusion in asthma vaccines. This concept is supported by the report that BCG vaccination is associated with a reduction in atopic diseases and increased production of IFN-γ in Japanese school-children (22). However, CpG ODN would have to be administered to young children, to prevent initial allergic sensitization, and some inherent problems may exist with this approach. As mentioned previously, predominant Th1 responses are associated with some autoimmune diseases, suggesting that there is a risk associated with CpG ODN administration and the resultant Th1 immune deviation in young children. Conceivably, this risk could be mitigated by administration of CpG ODN conjugated to specific allergens, thus limiting Th1 immune deviation to allergen-specific effects. One problem with this approach is that it is difficult to predict, beforehand, the allergens that would be expected to sensitize individual children, but, perhaps educated guesses based on family history and individual environmental exposures could be attempted with some effectiveness. An alternate approach would be to avoid administration of CpG ODN to children, and instead focus on efforts to suppress Th2-mediated allergies in adults, where allergic sensitizations could be better characterized. The studies showing that CpG ODN effectively inhibit allergic responses after allergic sensitization in murine models, as well as convert human cell populations derived from atopic individuals from a Th2 to a Th1 bias, suggest that this approach might be effective.

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