

DNA Vaccines: An Evolving Approach to the Treatment of Allergic Disorders

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ABSTRACT

Immunotherapy with protein allergens is effective in modulating the immune response and reducing symptoms in patients with allergic rhinitis and asthma. Unfortunately, the dose of allergen that can be administered to allergic asthmatic patients, who might most benefit from this form of therapy, is frequently limited because of reduced FEV₁ and safety concerns. Therefore, alternative strategies to identify ways to minimize the allergenicity of immunotherapy while improving the effectiveness are currently being investigated in animal models of asthma and in human subjects with allergic rhinitis. Several DNA-based methods of immunization (CpG DNA, CpG DNA conjugated to a protein allergen, and plasmid DNA) have shown promise in animal models of asthma, and some of these DNA-based therapies have entered phase I/II clinical trials. Furthermore, ongoing large-scale phase III clinical trials will be able to determine whether any of these DNA-based therapies are safe and effective to use in subjects with allergic rhinitis and asthma. (Allergy and Asthma Proc 26:195–198, 2005)

In general, therapeutic strategies in allergic inflammation have either focused on antagonizing a single downstream mediator (e.g., histamine, LTC₄), or alternatively

targeting an upstream cell (e.g., Th2 lymphocyte) which regulates the expression of multiple downstream cytokines and mediators. The advantage of targeting a single downstream mediator is the potential improved safety margin of such a therapy compared to targeting an upstream cell, which regulates the expression of multiple mediators and cytokines, some of which may be important to host defense. However, in asthma a therapy that inhibits multiple mediators and cytokines (e.g., corticosteroids) is likely to be much more effective than therapies that have targeted a single cytokine/mediator. This is likely due to the fact that in asthma multiple cells, cytokines, and mediators play an important role in disease expression. DNA-based vaccines represent a novel therapeutic strategy to inhibit the Th2 cell from expressing several cytokines important to the pathogenesis of asthma. Recent studies in animal models of asthma, as well as preliminary studies in humans, have investigated the therapeutic potential of several DNA-based vaccine strategies, including the use of immunostimulatory DNA sequences (CpG DNA), CpG DNA conjugated to an allergen protein, and DNA allergen gene vaccines.

CpG DNA AND INHIBITION OF Th2 IMMUNE RESPONSES

The ability of CpG DNA to inhibit Th2 cells, a driving force in the development of allergic inflammation, is the rationale for investigating its effectiveness as a novel therapy in asthma. The immunostimulatory activity of CpG DNA is contained in a six-basepair DNA sequence that follows the formula 5'-purine-purine-CpG-pyrimidine-pyrimidine-3'¹ (Fig. 1). This sequence of DNA does not code for a protein, but rather exerts its biologic function by binding to Toll-receptor 9 (TLR-9), which is expressed on cells of the innate immune system.² Thus, CpG DNA does not inhibit Th2 responses by binding to T cells (which do

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Figure 1. CpG DNA. The immunostimulatory activity of CpG DNA is contained in a six-base pair DNA sequence which follows the formula 5'-purine-purine-CpG-pyrimidine-pyrimidine-3.

not express TLR-9), but rather acts indirectly on T lymphocytes by inducing cells of the innate immune response (especially dendritic cells, which express high levels of TLR-9) to express cytokines, which inhibit the Th2 response. The immune response to CpG DNA is characterized by the expression of Th1 cytokines including IFN- γ , as well as IL-6, IL-10, IL-12, IL-18, and IFNs α and β .³ In addition to inducing Th1 responses, CpG DNA inhibits expression of Th2 cytokines in the lung including IL-4, IL-5, IL-9, and IL-13, while also reducing levels of expression of Th2 cytokine receptors such as the IL-4 receptor.³

CpG DNA IN MOUSE MODELS OF THE PREVENTION AND REVERSAL OF ASTHMA

Several studies have demonstrated that CpG DNA can inhibit Th2 cytokine responses, as well as eosinophilic inflammation and airway hyperreactivity to methacholine in mouse models of asthma.⁴ CpG DNA can prevent eosinophilic inflammation and airway hyperreactivity when administered before allergen inhalation challenge, and can also reverse eosinophilic airway inflammation and airway hyperreactivity when first administered at a time point when there was already significant eosinophilic airway inflammation.⁵ These studies suggest that CpG DNA, like corticosteroids, can be used to reverse an episode of allergen-induced airway inflammation. CpG DNA is effective in inhibiting eosinophilic airway inflammation when administered either systemically or mucosally to the airway, and this effect lasts for at least 4 weeks after a single systemic dose. CpG DNA inhibits generation of IL-5, which results in decreased production and release of eosinophils from the bone marrow.

COMPARISON OF CpG DNA AND CORTICOSTEROIDS IN MOUSE MODELS OF ASTHMA

As corticosteroids are the current best available anti-inflammatory therapy in asthma, studies have compared how effective CpG DNA is compared to corticosteroids in inhibiting allergic inflammatory responses in mouse models of asthma.⁵ Interestingly, in preventive models of asthma in mice, a single dose of CpG DNA has been shown to reduce airway eosinophilia as effectively as systemic administration of corticosteroids administered for 7 days.⁴ In mouse models in which therapy is first administered after the induction of airway hyperreactivity, CpG DNA decreases airway hyperreactivity as effectively as dexamethasone.⁵ The combination of CpG DNA and dexamethasone therapy is more effective than monotherapy with either agent alone in reducing airway hyperreactivity.⁵

CpG DNA AND MOUSE MODEL OF VIRUS-INDUCED ASTHMA

The ability of CpG DNA to inhibit virus-induced asthma has been examined in a mouse model of respiratory syncytial virus (RSV)-induced airway inflammation. Mice pretreated with CpG DNA express the anti-viral cytokines interferon- α , - β , and - γ in the lung and this is associated with significantly reduced RSV viral titers, peribronchial inflammation, and mucus secretion.⁶ Administration of CpG DNA to mice inhibits RSV replication within days of RSV infection *in vivo*, suggesting that its effect is primarily due to activation of innate immunity as opposed to adaptive immunity. Thus, CpG DNA therapy administered to prevent allergen-induced airway inflammation and airway hyperreactivity could have additional potential benefits in reducing the severity of RSV infections that are known precipitants of asthma.⁶

CpG DNA INHIBITS AIRWAY REMODELING IN MICE

Recent studies have demonstrated that CpG DNA can prevent airway remodeling in mice exposed to repetitive airway challenge with allergen.⁷ CpG DNA reduces the thickness of the peribronchial smooth muscle layer, accumulation of peribronchial myofibroblasts, and reduces peribronchial fibrosis (peribronchial collagen III and V deposition). In addition, CpG DNA significantly reduces bronchoalveolar lavage and lung levels of the pro-fibrotic cytokine TGF- β , suggesting that inhibition of TGF- β expression may account for the reduction in airway remodeling. TGF- β stimulates fibroblasts to produce extracellular matrix proteins (collagen, fibronectin), and decreases the production of enzymes that degrade the extracellular matrix (collagenase), with the net effect being to increase the production of extracellular matrix proteins. In subjects with asthma increased levels of TGF- β have been reported in BAL and biopsy specimens.⁸ TGF- β expression correlates with the degree of subepithelial fibrosis, and levels of TGF- β are significantly increased in patients with severe asthma who have prominent airway eosinophilic inflammation.⁹ Overall, these studies suggest that CpG DNA not only prevents Th2-mediated airway inflammation in response to acute allergen challenge, but also TGF- β expression and airway remodeling associated with chronic allergen challenge.

POTENTIAL FOR CpG DNA-BASED THERAPY IN HUMAN ALLERGY AND ASTHMA

In humans, as in mice, TLR-9 is the receptor for CpG DNA, and thus cell types that express TLR-9 (dendritic cells) respond to CpG DNA stimulation.² CpG DNA di-

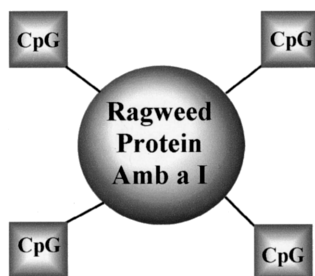


Figure 2. CpG DNA protein allergen conjugate. AIC is an example of a conjugate of CpG DNA and a protein allergen that has been used in clinical trials in subjects with allergic rhinitis. AIC comprises Amb a 1 (the major ragweed protein allergen) conjugated to four CpG DNA sequences.

rectly activates human dendritic cells (plasmacytoid dendritic cell subset) to express increased levels of co-stimulatory molecules and cytokines. In studies of mononuclear cells derived from dust mite allergic individuals CpG DNA inhibited Der p 1 induced IL-4 production and induced Der p 1 IFN- γ production.¹⁰ At present there are no published results of studies investigating the safety and efficacy of CpG DNA *in vivo* in human allergy and asthma.

CONJUGATION OF CpG DNA AND PROTEIN ALLERGEN TO INHIBIT ALLERGIC RESPONSES

The ability of CpG DNA conjugated to a protein antigen to induce a Th1 immune response is 100-fold higher than that induced by a mixture of equivalent amounts of CpG DNA and protein antigen.¹¹ The enhanced immunomodulatory activity of the CpG DNA protein antigen conjugate is hypothesized to be due to the subcutaneously injected CpG DNA and conjugated protein antigen being engulfed by the same antigen presenting cell, whereas when they are not conjugated CpG DNA and the protein antigen will not always end up in the same antigen presenting cell. Studies have demonstrated that dendritic cells bound >100-fold more CpG DNA conjugated to antigen compared to CpG DNA alone.¹² CpG DNA conjugated to antigen therefore potentiates the ability of dendritic cells to promote Th1 cellular immune responses.¹² In a mouse model of asthma either airway mucosal delivery or intradermal administration of a CpG DNA allergen protein conjugate significantly reduces airway hyperreactivity to methacholine for at least 2 months.¹³ The CpG DNA allergen protein conjugate is 100-fold more efficient than the unconjugated mixture in reducing airway eosinophilia and bronchial hyperreactivity.¹³

POTENTIAL FOR CpG DNA ALLERGEN PROTEIN CONJUGATE-BASED THERAPY IN HUMAN ALLERGY AND ASTHMA

In studies of human mononuclear cells, a CpG DNA ragweed protein conjugate (Fig. 2) was more effective at enhancing IFN- γ production and inhibiting IL-4 produc-

tion compared to CpG DNA alone.¹⁴ The CpG DNA Amb a 1 conjugate induces significantly less basophil histamine release compared to ragweed alone.¹⁵ Preliminary studies suggest that a CpG DNA Amb a 1 conjugate is less allergenic than the allergen protein based on immediate hypersensitivity skin test reactivity,¹⁵ and that a six-injection regimen of the CpG DNA Amb a 1 conjugate reduces symptoms of allergic rhinitis in ragweed-sensitized human subjects followed through the ragweed season.^{15,16} Studies of nasal mucosal biopsies in subjects with ragweed-induced allergic rhinitis have demonstrated a significant decrease in nasal mucosal eosinophils and Th2 cytokines (*i.e.*, IL-4), which was associated with a significant increase in Th1 cytokines (IFN- γ) in CpG DNA Amb a 1 conjugate-treated compared to placebo-treated subjects.¹⁷ However, although these small scale phase I/II studies have suggested the potential of this therapeutic approach, further large-scale studies are needed to demonstrate whether this approach is safe and effective in the therapy of human allergy and asthma.

ALLERGEN GENE VACCINES AND ASTHMA

Gene vaccines (also known as plasmid DNA) have been investigated in infectious diseases, cancer, and allergy and asthma. The plasmid DNA (pDNA) is a circular strand of DNA, which in the case of allergy immunotherapy is engineered to contain the DNA coding region of an allergen of interest (*e.g.*, dust mite) linked to a promoter that drives the expression of the allergen coding region. Gene vaccines encoding an allergen are able to inhibit IgE and Th2 immune responses, as well as airway hyperreactivity in animal models of asthma.^{18,19} CpG DNA sequences in the noncoding plasmid backbone of the plasmid DNA acts as a Th1 adjuvant and promote Th1 rather than Th2 responses to the encoded allergen. In a rat model of asthma, immunization with a pDNA encoding the Der p 5 allergen inhibited the IgE response, histamine release into bronchoalveolar lavage fluid, and bronchial hyperreactivity following challenge with aerosolized dust mite allergen Der p 5.¹⁹ The response to the gene vaccine is antigen-specific, as the rats immunized with pDNA encoding the Der p 5 allergen were protected against Der p 5 allergen challenge, but not against a different allergen, *i.e.*, ovalbumin challenge.¹⁹ At present there are no published studies of the safety or efficacy of DNA vaccines encoding allergens in humans with allergy or asthma.

CONCLUSION

A variety of DNA-based vaccine (CpG DNA, CpG DNA allergen protein conjugate, allergen gene vaccine) strategies have demonstrated the potential to prevent or reverse allergen-induced airway inflammation, Th2 cytokine responses, mucus production, and airway hyperreactivity in animal models of asthma. Further ongoing studies in human subjects will determine whether any of these

strategies are safe and effective in the therapy of patients with asthma and allergic rhinitis.

REFERENCES

1. Yamamoto S, Yamamoto T, Kataoka T, et al. Unique palindromic sequences in synthetic oligonucleotides are required to induce IFN and augment IFN-mediated natural killer cell activity. *J Immunol* 148:4072–4076, 1992.
2. Krieg AM. CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol* 20:709–760, 2002.
3. Spiegelberg HL, Horner AA, Takabayashi K, and Raz E. Allergen-immunostimulatory oligodeoxynucleotide conjugate: A novel allergoid for immunotherapy. *Curr Opin Allergy Clin Immunol* 2:547–551, 2002.
4. Broide D, Schwarze J, Tighe H, et al. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J Immunol* 161:7054–7062, 1998.
5. Ikeda RK, Nayar J, Cho JY, et al. Resolution of airway inflammation following OVA inhalation: Comparison of ISS DNA and corticosteroids. *Am J Resp Cell Mol Biol* 28:655–663, 2003.
6. Cho J, Miller M, Baek K, et al. Immunostimulatory DNA sequences inhibit respiratory syncytial viral load, airway inflammation, and mucus secretion. *J Allergy Clin Immunol* 108:697–702, 2001.
7. Cho JY, Miller M, Baek KJ, et al. Immunostimulatory DNA inhibits TGF- β expression and airway remodeling. *Am J Resp Cell Mol Biol* 30:651–661, 2004.
8. Davies DE, Wicks J, Powell RM, et al. Airway remodeling in asthma: New insights. *J Allergy Clin Immunol* 111:215–225, 2003.
9. Flood-Page P, Menzies-Gow A, Phipps S, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 112:1029–1036, 2003.
10. Bohle B, Jahn-Schmid B, Maurer D, et al. Oligodeoxynucleotides containing CpG motifs induce IL-12, IL-18, and IFN-gamma production in cells from allergic individuals and inhibits IgE synthesis in vitro. *Eur J Immunol* 29:2344–2353, 1999.
11. 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.
12. Shirota H, Sano K, Hirasawa K, et al. Novel roles of CpG oligodeoxynucleotides as a leader for the sampling and presentation of CpG tagged antigen by dendritic cells. *J Immunol* 167:66–74, 2001.
13. Santeliz JV, Van Nest G, Traquina P, et al. Amb a 1-linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma. *J Allergy Clin Immunol* 109:455–462, 2002.
14. Marshall JD, Abtahi S, Eiden J, et al. Immunostimulatory sequence DNA linked to the Amb a 1 allergen promotes Th1 cytokine expression while downregulating Th2 cytokine expression in PBMC's from human patients with ragweed allergy. *J Allergy Clin Immunol* 108:191–197, 2001.
15. Creticos PS, Eiden JJ, Balcer SL, et al. Immunostimulatory oligodeoxynucleotides conjugated to Amb a 1: Safety, skin test reactivity, and basophil histamine release. *J Allergy Clin Immunol* 105:S70, 2000.
16. Creticos PS, Eiden JJ, Broide DH, et al. Immunotherapy with immunostimulatory oligonucleotides linked to purified ragweed Amb a 1 allergen: Effects on antibody production, nasal allergen provocation, and ragweed seasonal rhinitis. *J Allergy Clin Immunol* 109:743, 2002.
17. Tulic MK, Fiset PO, Christodoulouopoulos P, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol* 113:235–241, 2004.
18. Raz E, Tighe H, Sato Y, et al. Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization. *Proc Natl Acad Sci USA* 93:5141–5145, 1996.
19. Hsu CH, Chua KY, Tao MH, et al. Immunoprophylaxis of allergen-induced immunoglobulin E synthesis and airway hyperresponsiveness in vivo by genetic immunization. *Nat Med* 2:540–544, 1996. □

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