

Gee whiz: CpG DNA allergy therapy!

Key words: Immunotherapy

Immunotherapy for allergic diseases by injection of aqueous allergen extracts to desensitize reactivity is a procedure used since early in the 20th century. Controlled studies support use of immunotherapy for allergic rhinitis,¹ for systemic anaphylaxis induced by insect stings,² and for many cases of allergic asthma.^{3,4} Postulated mechanisms for therapeutic effects have been revised successively. Prior emphasis on induction of IgG antibodies that block allergen from binding to IgE on mast cells⁵ has given way to ideas about inhibition of allergy effector T_H2 cells^{6,7} and IgE by suppressive cytokines such as IL-10^{8,9} or via IFN- γ and IL-12 induced in counterbalancing T_H1 cells.^{8,10-13}

Revision of materials administered for clinical allergen immunotherapy has included alum precipitates, pyridine extracts, formalin-denatured allergoids, and glutaraldehyde polymers,¹ but these generally lost immunogenicity at the price of lessened allergenicity. Now the latest potential method concerns possible administration of allergens together with adjuvant DNA oligonucleotides, which may preferentially induce counterbalancing T_H1 responses¹⁴ while also having reduced allergenicity.

This is the subject of an important article in this issue of *The Journal of Allergy and Clinical Immunology* (p 124).¹⁵ These adjuvant DNA immunostimulatory sequences (ISS) favor T_H1 responses by containing unmethylated cytosine and guanosine dinucleotide repeat motifs in DNA with adjuvant properties. Such CpG motifs are found in bacteria and are present at low frequency in vertebrates and thus stimulate elements of antibacterial innate immunity, such as antigen-presenting dendritic cells and natural killer cells that produce IL-12, IFN- α/β , tumor necrosis factor- α , and IL-18 (IFN- γ inducing factor)^{16,17} to bias responses to the associated allergen in a T_H1 direction. CpG DNA may potentially suppress T_H2 responses, including IgE to the allergen, via induced IFN- γ or IL-10,^{18,19} or via CD8 cells,²⁰ which could be related to previously described immunotherapy

Abbreviations used

CpG: Unmethylated cytosine and guanosine dinucleotide repeat motif in DNA with adjuvant properties
ISS: Immunostimulatory sequences

suppressor cells.²¹ CpG DNA sequences can have immunostimulatory properties when administered in different ways, for example, when encoded with an allergen complementary DNA sequence for production of the allergen protein in the host,²² when given alone^{23,24} or together with the allergen,^{25,26} and even when administered orally.²⁷ Recently, this has made for an exploding literature that promises to produce a wide dissemination of this CpG approach into virtually all conceivable situations (Fig 1). Importantly, the study by Tighe et al, in which synthetic ISS were used, has introduced the novel approach of conjugating the immunostimulatory DNA sequences to purified Amb a 1 protein, the dominant ragweed allergen, to use for immunotherapy in mice.¹⁵

Injection of Amb a 1 chemically linked to ISS CpG indeed led to T_H1-biased responses to Amb a 1, with production of T_H1 IFN- γ -dependent IgG_{2a} and IgG_{2b} antibody isotypes. Moreover, a key finding was that the conjugates inhibited IgE production to Amb a 1, even in mice that were immunized previously with Amb a 1 in alum, which as usual induced IgE. Therefore therapeutic conjugates of ragweed allergen linked to CpG DNA inhibited IgE responses in mice that were previously immunized to produce IgE by virtue of their genetic makeup and the adjuvants used. This is analogous to patients whom allergists treat with desensitization, that is, genetically susceptible individuals who were immunized previously and are actively producing IgE. Thus there is promise that if this CpG-linked form of immunotherapy is extended to the clinical arena, there might be a great anti-allergic benefit.

There also is another potential virtue to therapy with CpG-allergen conjugates. Anaphylactic reactivity usually greatly limits dose-progressive allergen immunotherapy with native proteins. In preliminary clinical studies, these authors studied the potential allergenicity of the Amb a 1-CpG conjugates. The results show that conjugates had significantly less ability to bind IgE²⁷ and were less able to trigger basophil histamine release via specific anti-Amb a 1 IgE, probably because of attached interfering DNA. Also, there was nearly 200-fold less ability to produce immediate skin test responses.²⁸ These results suggest that future immunotherapy with conjugates of allergens and

From Section of Allergy and Clinical Immunology, Department of Medicine, Yale University School of Medicine.

Received for publication Apr 24, 2000; revised May 3, 2000; accepted for publication May 3, 2000.

Reprint requests: Philip W. Askenase, MD, Section of Allergy and Clinical Immunology, Department of Medicine, Yale University School of Medicine, 333 Cedar St, LCI 904, New Haven, CT 06520-8013.

J Allergy Clin Immunol 2000;106:37-40.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000/\$12.00 + 0 1/1/108363

doi:10.1067/mai.2000.108363



FIG 1. Blossoming CpG therapy promises to soon show up in virtually all conceivable situations.

CpG-containing DNA ISS might simultaneously induce protective T_H1 responses to counterbalance allergy-provoking T_H2 and IgE responses and might simultaneously trigger less allergic reactivity. The effects of the conjugates were quite specific. DNA conjugates without CpG sequences were not protective, and even coinjection of unlinked Amb a 1 with the identical adjuvant CpG ISS was much less effective. Thus the key here was a requirement for conjugate linkage, suggesting that the ISS and allergen need to be focused in the same antigen-presenting cell,

which makes sense. A further important point was that conjugates also induced high-titer specific IgG antibodies in rabbits and monkeys, providing further hope that the results obtained in mice might also occur in humans.

Thus overall, these CpG conjugates may offer more effective and simultaneously safer allergen immunotherapy than heretofore possible. However, an important point in analyzing the potential use of CpG conjugates in allergen immunotherapy concerns whether the induced augmentation of T_H1 responses will actually be suppres-

sive of allergic diseases in humans. Although it is clear from basic studies, particularly in mice, that T_H1 IFN- γ -dominated responses are suppressive of T_H2 and particularly IgE responses and result in protection from manifestations of allergy, there is only limited evidence that this paradigm applies to human allergic diseases. Noteworthy are studies that do suggest that accompanying the benefit of conventional allergen immunotherapy, there is activation of T_H1 cells to stimulate production of IFN- γ and possibly IL-12 and simultaneous inhibition of IL-4 and IL-5. However, in some studies immunotherapy inhibits allergic disease and T_H2 cytokines without apparent induction of T_H1 IFN- γ or IL-12.²⁹⁻³² Importantly, patients undergoing venom immunotherapy for anaphylactic stinging insect hypersensitivity become protected, but not via counterbalancing T_H1 effects. Instead, this immunotherapy induces suppressor or regulatory T cells, and subsequently, macrophages that produce suppressive IL-10, which inhibits T_H2 proliferation, cytokine production, and IgE responses^{7,8} by inhibiting CD28 tyrosine phosphorylation that hampers co-stimulatory signaling of allergy-effector T_H2 cells.³³

Thus if stimulation of T_H1 responses is not universally beneficial in allergen immunotherapy, then the CpG approach may only apply to some patients or to some allergens, making its general use difficult. On the other hand, CpG conceivably could stimulate inhibitory IL-10-producing T_{reg} cells and IL-10-producing cells. Another unresolved point is that immune responses are most easily manipulated at the beginning, whereas established responses, which surely pertain to patients with existing allergy, are more difficult to alter. Although this point was addressed in the study by Tighe et al¹⁵ by showing inhibition of a primed IgE response during boosting, most successful studies of CpG therapy of allergic disease models have started treatment at induction of the primary response or before the secondary boost,³⁴ whereas only a few studies have reported significant inhibition of thoroughly established allergic responses.³⁵

Furthermore, there is a potentially significant downside to T_H1 enhancing immunotherapy in asthma. Some asthma models in mice have failed to show T_H1 inhibition of T_H2 airway responses.³⁶ In fact, some investigators have stressed that induction of allergen-specific T_H1 cells could produce a synergistic and also eosinophil-rich airway inflammation, which might make things worse.³⁷⁻³⁹ However, this is controversial, because other groups working with mice that were similarly immunized with ovalbumin reported beneficial T_H1 effects via IFN- γ reduction of airway mucus production and eosinophilia.⁴⁰ Further, in a rat model of ovalbumin allergic asthma with robust actual airway resistance measurements of hyperreactivity, it was shown that allergen-specific T_H1 T-cell lines that produced mild inflammation without hyperreactivity on their own, when given together with asthma-promoting T_H2 lines, were potent suppressors of bronchoalveolar lavage eosinophils and airway IL-4 messenger RNA expression.⁴¹ Most importantly, the T_H1

lines clearly inhibited airway hyperreactivity that was produced by the T_H2 T-cell lines, and in a dose-response and antigen-specific fashion.⁴¹ Another caution is that enhanced T_H1 responses could induce IFN- γ -dependent complement-activating antibody isotypes, leading to immune complex-type inflammation.

Thus although the jury is still out on the possible effectiveness of T_H1 -augmented immunization for human allergic diseases and on the beneficial versus potentially deleterious effects of such therapy, particularly in asthma, and on the ability of CpG conjugates to inhibit established allergy, it is anticipated that this new CpG technology will proceed into human trials.

Finally, one must wonder whether we have been here before. Ideas of CpG adjuvant sequences in cDNA vaccines are new. However, for many years previously, allergists used bacterial vaccines of heat-killed organisms as adjuvants with allergen immunotherapy⁴² without confirmed proof of efficacy or knowledge of mechanisms or constituent substances. In hindsight, bacterial vaccine undoubtedly contained CpG adjuvant sequences that may have produced some benefit. Thus CpG sequence allergen immunotherapy may represent a more advanced molecular adjuvant version, which may provide more benefit and safety by use of defined substances.

Still, important questions remain. Will we need to administer CpG conjugates to the separate molecules of each allergen mixture and thus need repeated, prolonged, complex, and expensive regulatory processes in each case? Alternatively, can ways be found to increase the benefit of CpG ISS given unlinked but together with diverse allergens, as is often effective,^{25,26} to still focus on the same antigen-presenting cell and thus require less regulatory processing? These are some of the problems that this area of great promise will have to face in an interesting future.

Philip W. Askenase, MD

Professor of Medicine

Chief, Section of Allergy and Clinical Immunology

Department of Medicine

Yale University School of Medicine

New Haven, CT 06520-8013

REFERENCES

1. Nelson HS. Immunotherapy for inhalant allergens. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, editors. *Allergy: principles and practice*. 5th ed. St Louis: Mosby; 1998. p. 1050-62.
2. Yunginger JW. Insect allergy. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse WW, editors. *Allergy: principles and practice*. 5th ed. St. Louis: Mosby; 1998. p. 1063-72.
3. Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? *Am J Respir Crit Care Med* 1995;151:969-74.
4. Creticos PS, Reed CE, Norman PS, Khoury J, Adkinson NF Jr, Buncher CR, et al. Ragweed immunotherapy in adult asthma. *N Engl J Med* 1996; 334:501-6.
5. Reid MJ, Moss RB, Hsu YP, Kwasnicki JM, Commerford TM, Nelson BL. Seasonal asthma in northern California: allergic causes and efficacy of immunotherapy. *J Allergy Clin Immunol* 1986;78:590-600.
6. Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin-4 production in CD4⁺ T cells from allergic individuals. *J Exp Med* 1993;178:2123-30.
7. Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998;102:157-64.

8. Bellinghausen I, Metz G, Enk AH, Christmann S, Knop J, Saloga J. Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects. *Eur J Immunol* 1997;27:1131-9.
9. Akdis CA, Blesken T, Akdis M, Wutrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998;102:98-106.
10. Jutel M, Pichler WJ, Skrebic D, Urwyler A, Dahinden C, Muller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. *J Immunol* 1995;154:4187-94.
11. Varney VA, Hamid QA, Gaga M, Ying S, Jacobson M, Frew AJ, et al. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993;92:644-51.
12. Lack G, Nelson HS, Amran D, Oshiba A, Jung T, Bradley KL, et al. Rush immunotherapy results in allergen-specific alterations in lymphocyte function and interferon- γ production in CD4⁺ T cells. *J Allergy Clin Immunol* 1997;99:530-8.
13. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4⁺ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon- γ . *J Allergy Clin Immunol* 1996;97:1356-65.
14. Chu RS, Targoni OS, Krieg AM, Lehmann PV, Harding CV. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (Th1) immunity. *J Exp Med* 1997;186:1623-31.
15. Tighe H, Takabayashi K, Schwartz D, Van Nest G, Tuck S, Eiden JJ, 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* 2000;106:124-34.
16. Hartmann G, Weiner GJ, Krieg AM. CpG DNA: a potent signal for growth, activation, and maturation of human dendritic cells. *Proc Natl Acad Sci U S A* 1999;96:9305-10.
17. Kranzer K, Bauer M, Lipford GB, Heeg K, Wagner H, Lang R. CpG-oligodeoxynucleotides enhance T cell receptor-triggered interferon-gamma production and up-regulation of CD69 via induction of antigen-presenting cell-derived interferon type I and interleukin-12. *Immunology* 2000;99:170-8.
18. Horner A, Takabayashi K, Burger J, Ronaghy A, Cinman N, Spiegelberg H, et al. Immunostimulatory sequence oligodeoxynucleotide induces an innate type 1 cytokine response from human PBMCs and inhibits IL-4 dependent CD23 expression and IgE production [abstract]. *J Allergy Clin Immunol* 2000;105:S69.
19. Marshall JD, Eiden JJ, Kagey-Sobotka A, Creticos PS, Lichtenstein LM, Van Nest G. ISS conjugated to Amb a 1 allergen promotes a Th2 to Th1 switch in PBMC from ragweed-allergic humans [abstract]. *J Allergy Clin Immunol* 2000;105:S76.
20. Cho HJ, Nguyen M-D, Chiu Y, Raz E. T helper-independent activation of antigen-specific cytotoxic lymphocytes and exogenous antigen presentation by immunostimulatory sequence DNA-based vaccines [abstract]. *J Allergy Clin Immunol* 2000;105:S163.
21. Tamir R, Castracane JM, Rocklin RE. Generation of suppressor cells in atopic patients during immunotherapy that modulate IgE synthesis. *J Allergy Clin Immunol* 1987;79:591.
22. Hus C-H, Chua K-Y, Tao M-H, Lai Y-L, Wu H-D, Huang S-K, et al. Immunoprophylaxis of allergen-induced immunoglobulin E synthesis and airway hyperresponsiveness in vivo by genetic immunization. *Nat Med* 1996;2:540-4.
23. Broide D, Schwarze J, Tighe H, Gifford T, Nguyen MD, Malek S, et al. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J Immunol* 1998;161:7054-62.
24. Bohle B, Jahn-Schmid B, Maurer D, Kraft D, Ebner C. Oligodeoxynucleotides containing CpG motifs induce IL-12, IL-18 and IFN-gamma production in cells from allergic individuals and inhibit IgE synthesis in vitro. *Eur J Immunol* 1999;29:2344-53.
25. Shiota 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* 2000;22:176-82.
26. Jahn-Schmid B, Widermann U, Bohle B, Repa A, Kraft D, Ebner C. Oligodeoxynucleotides containing CpG motifs modulate the allergic Th2 response of BALB/c mice to Bet v 1, the major birch pollen allergen. *J Allergy Clin Immunol* 1999;104:1015-23.
27. Kline JN, Businga TR, Jain V, Hussein I. Oral administration of CpG oligodeoxynucleotides provide protection against inflammation in asthma [abstract]. *J Respir Clin Care Med* 2000;161:A21.
28. Creticos PS, Eiden JJ, Balcer SL, Van Nest G, Kagey-Sobotka A, Tuck SF, et al. Immunostimulatory oligodeoxynucleotides conjugated to Amb a 1: safety, skin test reactivity, and basophil histamine release [abstract]. *J Allergy Clin Immunol* 2000;105:S70.
29. O'Brien RM, Byron KA, Varigos GA, Thomas WR. House dust mite immunotherapy results in a decrease in Der p 2-specific IFN-gamma and IL-4 expression by circulating T lymphocytes. *Clin Exp Allergy* 1997;27:46-51.
30. Meissner N, Kochs S, Coutelle J, Kussebi F, Baumgarten C, Lowenstein H, et al. Modified T cell activation pattern during specific immunotherapy (SIT) in cat allergic patients. *Clin Exp Allergy* 1999;29:618-25.
31. 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* 1999;104:1258-64.
32. O'Brien RM, Xu H, Rolland JM, Byron KA, Thomas WR. Allergen-specific production of interferon- γ by peripheral blood mononuclear cells and CD8 T cells in allergic disease and following immunotherapy. *Clin Exp Allergy* 2000;30:333-40.
33. Joss A, Akdis M, Faith A, Blaser K, Akdis CA. Interleukin-10 directly acts on T cells by specifically altering the CD28 costimulatory pathway. In Press, 2000.
34. 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* 1999;162:6284-93.
35. Kline JN, Waldschmidt TJ, Businga TR, Lemish JE, Weinstock JV, Thorne PS, et al. Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. *J Immunol* 1998;160:2555-9.
36. Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. *J Clin Invest* 1999;103:175-83.
37. Randolph DA, Carruthers CJ, Szabo SJ, Murphy KM, Chaplin DD. Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. *J Immunol* 1999;162:2375-83.
38. Randolph DA, Stephens R, Carruthers CJ, Chaplin DD. Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J Clin Invest* 1999;104:1021-9.
39. Wild JS, Sigounas A, Sur N, Siddiqui MS, Alam R, Kurimoto M, et al. IFN- γ inducing factor (IL-18) increases allergic sensitization serum IgE, Th2 cytokines and airway eosinophilia in a mouse model of allergic asthma. *J Immunol* 2000;164:2701-10.
40. Cohn L, Homer RJ, Niu N, Bottomly K. T helper 1 cells and interferon gamma regulate allergic airway inflammation and mucus production. *J Exp Med* 1999;190:1309-18.
41. Huang T-J, MacAry PA, Salmon M, Wilke T, Askenase PW, Kemeny DM, et al. Adoptive transfer of ovalbumin-specific Th2 lymphocytes induce bronchial responsiveness and eosinophilia; inhibition by ovalbumin-specific Th1 lymphocytes [abstract]. *Am J Respir Crit Care Med* 1999;159:A509.
42. Mueller HL, Lanz M. Hyposensitization with bacterial vaccine in infectious asthma. A double-blind study and a longitudinal study. *JAMA* 1969;26:1379-83.