

New approaches in immunotherapy: allergen vaccination with immunostimulatory DNA

Peter Socrates Creticos, MD^{a,*}, Yi-Hsing Chen, MD^b,
John T. Schroeder, PhD^a

^a*Division of Allergy and Clinical Immunology, Department of Medicine,
Johns Hopkins Asthma and Allergy Center, Johns Hopkins University, 5501 Hopkins Bayview Circle,
Room 2B, 71, Baltimore, MD 21224, USA*

^b*Paichung Veterans General Hospital, N0160 Taichung Harbor Road-Section 3,
40705 Taichung, Taiwan*

Allergen immunotherapy, first introduced in the early part of the twentieth century, remains a cornerstone of the practicing allergists' approach to the treatment of allergic respiratory diseases. It is comprised of a time-honored approach that aims to modulate the immune response and attenuate or eliminate symptoms through administration of increasing doses of an extract that is comprised of the aeroallergens to which the patient is allergic. Controlled clinical trials have demonstrated therapeutic efficacy and detailed the favorable immunologic changes associated with allergen immunotherapy for allergic rhinitis, asthma, and venom sensitivity [1–7].

Despite its success, conventional immunotherapy is saddled with a number of encumbrances. Impediments include the need for frequent dosing over years, which impacts patient compliance; the need to administer a relatively large dose of the immunizing agent to achieve control of symptoms; and the potential for clinically significant allergic reactions to the treatment. Considerable effort has been devoted to developing improved therapeutic vaccines for allergic diseases to: (1) improve efficacy, (2) decrease the time required to achieve effect, (3) reduce inconvenience and improve compliance with immunization regimens, and (4) enhance safety.

Recognizing that allergen dose is limited by the potential for systemic reactions to the respective allergens, efforts largely have been directed at decreasing

* Corresponding author.

E-mail address: pcretic@jhmi.edu (P.S. Creticos).

the allergenicity (ie, the potential for inducing an allergic reaction) of the immunizing antigens while preserving their immunogenicity (ie, the ability to induce a beneficial immunologic response). Although various chemical modifications of allergens have been attempted over the past several decades, the end result has been that allergenicity and immunogenicity decreased or increased in tandem, resulting in no improved risk–benefit ratio.

An allergenic vaccine that could reduce allergenicity, maintain immunogenicity, and be given in a few doses would have important therapeutic implications, as millions of patients with poorly controlled allergic rhinitis and asthma would be candidates for such a form of immunomodulation.

This article addresses a specific adjuvant approach to immunotherapy in which highly active immunostimulatory phosphorothioate oligodeoxyribonucleotide (ISS-ODN) moieties are linked to the principal allergenic moiety of a relevant aeroallergen (eg, ragweed Amb a 1) [8,9]. This adjuvant approach may prove to be highly effective at directing the immune response toward a more favorable T helper cell type 1 (Th1) phenotypic expression to counterbalance the untoward Th2-driven proinflammatory allergic process.

The allergic diathesis

To develop improved materials for immunotherapy, it is important to recognize the pathophysiology that underpins the allergic diathesis. On allergen exposure, susceptible individuals develop acute allergic responses that are indicative of IgE-dependent mast cell activation, which results in the release of preformed chemicals (histamine, various enzymes), newly generated inflammatory mediators of the arachidonic acid pathway (leukotrienes, prostaglandins), and cytokine molecules [10–12]. Tangential to this IgE-mediated allergic reaction, allergen also is processed by antigen-presenting cells that are displayed in association with class II human leukocyte antigen molecules and presented to T-lymphocytes. If appropriate, co-stimulatory signals are induced, resulting in T-cell activation, induction of Th2 cell development, recruitment to the site of allergic inflammation, and subsequent elaboration of proinflammatory cytokines (eg, interleukin 4 [IL-4], IL-5, IL-13) [13–16]. These cytokines help to amplify and sustain the overall response by regulating IgE synthesis, eosinophil survival, eosinophil migration, and mucus production, resulting in airway inflammation. The identification of transcription factors controlling Th1 and Th2 development further support this overall hypothesis, in that GATA3 (Th2-associated) is overexpressed in the asthmatic airway, whereas T-bet (Th1-associated) is underexpressed [17,18].

Mechanism of conventional immunotherapy

The authors' allergy group at Johns Hopkins took an early lead in examining the mechanisms by which allergen immunotherapy affects clinical improvement

in patients with allergic disease. These studies demonstrated that immunization initially resulted in production of IgG and IgE antibodies by antigen-specific B cells. With continuing immunotherapy, IgG levels further increased and plateaued, whereas antigen-specific IgE titers gradually declined toward pretreatment levels and were not boosted by subsequent environmental exposure to the allergen [19–22]. In conventional immunotherapy, induction of IgG antibodies is a predictor of clinical success, albeit clinical benefit is not likely to be achieved until the immunizing doses are large enough to risk anaphylaxis.

Various challenge models have been used as tools with which to evaluate the effects of immunotherapy. Specific nasal allergen provocation and nasal biopsy studies of patients with pollen sensitivity have been used to demonstrate that immunotherapy shifts the Th2–Th1 paradigm. Patients who successfully were immunized demonstrated attenuation of the acute challenge response (less inflammatory mediator release in nasal secretions) and an abrogation of the late-phase allergic reaction (decreased eosinophil and basophil migration and mediator release) [23–26]. Nasal biopsies of patients with rhinitis who were immunized against grass pollen and subsequently underwent challenge with grass pollen extract showed a significant increase in mRNA for specific Th1 cytokines (interferon γ [IFN- γ], IL-12) and a significant reduction in allergen-induced accumulation of total numbers of CD4⁺ T cells and eosinophils in the nasal mucosa. These techniques have resulted in little down-regulation in the expression of cytokines that are hallmarks of Th2 responses (eg, IL-4, IL-5) [27–30].

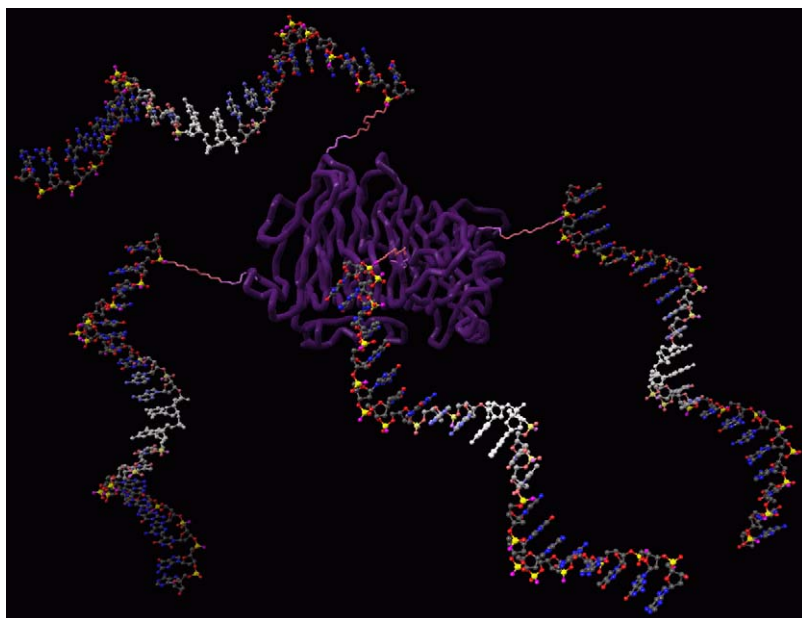


Fig. 1. AIC showing four molecules of ISS linked to purified ragweed Amb a 1.

Tangential to these observations, Secrist et al [31] cultured peripheral blood mononuclear cells from allergic patients receiving maintenance grass-pollen immunotherapy and demonstrated a significant decrease in allergen-induced IL-4 synthesis when these cells were exposed to allergen *in vitro*.

These collective studies demonstrate that immunotherapy has the potential to down-regulate the immediate-phase allergic reaction and T-cell-mediated late-phase responses; however, allergen dose is limited by the potential for severe systemic reactions in sensitized patients. At issue is the ability to administer a dose that is large enough to induce the desired therapeutic changes without causing intolerable allergic side effects. Raz et al [8] developed a novel immunostimulatory DNA construct termed AIC (Dynavax Technologies). This experimental vaccine is comprised of specific immunostimulatory oligodeoxyribonucleotide sequences (ISSs) that are conjugated to the principal allergenic moiety of ragweed (Amb a 1; Fig. 1). AIC has been shown to induce an enhanced, ragweed-specific, Th1-type response in mice, compared with Amb a 1 alone or Amb a 1 plus alum [32].

Background for adjuvant approaches

Various molecules, including lipopolysaccharides, aluminum hydroxide salts, and Freund's adjuvant, have been shown to possess immunostimulatory properties that can enhance the response to antigen. Various research laboratories have attempted to capitalize on this observation to improve a vaccine's immunogenicity through an enhanced adjuvant effect. In their study of the active components of Freund's adjuvant, Tokunaga et al [33–35] made the initial discovery of the adjuvant effect of bacterial DNA. Krieg et al [36] identified unmethylated CG dinucleotides, termed CpG motifs, as the moieties responsible for the immunostimulatory effect of bacterial DNA. These immunostimulatory properties are unique to certain bacterial DNAs and are absent in vertebrate DNA. This uniqueness is caused by the higher frequency of unmethylated CpG motifs in bacterial DNA as contrasted to vertebrate DNA. Without these differences, the immunostimulatory activity would be abolished [34,37,38].

The effects of bacterial DNAs can be mimicked using synthetic oligodeoxyribonucleotides (ODN), allowing a more accurate definition of the bacterial DNA immunostimulatory sequences (Box 1). Early research studies identified optimal ISSs containing palindromic hexamers based on the general formula of 5'-purine-purine-CG-pyrimidine-pyrimidine-3' (eg, 5'-GACGTC-3', 5'-AGCGCT-3', 5'-AACGTT-3') [36]. More recent work has extended this finding to the identification of different classes of CpG ODN based on their ability to preferentially activate plasmacytoid dendritic cells (PDCs), natural killer (NK) cells, or B cells. CpG-A ODN contains phosphodiester backbones and is effective at inducing interferon α production from PDCs and activating NK cells. CpG-B ODN possesses phosphorothioate backbones that enhance B-cell proliferation and immunoglobulin production, but is less capable of activating PDCs and NK

Box 1. Synthetic oligodeoxyribonucleotide immunostimulatory sequences

Believed to be taken up by professional antigen-presenting cells (macrophages, dendritic cells)

Resulting in secretion of specific co-stimulatory factors (IL-12, IL-18, TNF- α , IFN- α , IFN- β)

Activating a variety of immune effector cells (T cells, NK cells) and driving the adaptive immune response toward a Th1 phenotype

Inducing a potent humoral adjuvant effect

Enhanced immunostimulatory activity observed if ISS is coupled to allergen (AIC)

cells [39,40]. CpG-C represents a new class of ODN, possesses characteristics of the A and B classes, and is capable of activating B cells and PDCs [41,42].

Immunostimulatory oligodeoxyribonucleotide sequences induce Th1-type response and inhibit Th2-type immune response

Research has shown that CpG acts through toll-like receptor (TLR) 9 [43]. The association of CpG with TLR9 occurs intracellularly within lysosomes [44]; this interaction induces signals through the myeloid differentiation factor 88 pathway, eventually activating nuclear factor κ B (NF- κ B), much like what has been described for other TLR1 ligand interactions [45]. TLR9 was found to be expressed predominantly on human PDCs, basophils, and B cells, although some cell types have yet to be tested [46,47]. CpG-A ODN strongly stimulates PDCs to produce high amounts of IFN- α and IL-12, which activate IFN- γ -producing NK cells and $\gamma\delta$ T cells that drive immune cells toward Th1 responses (Fig. 2) [40,48–51].

Low concentrations of CpG ODN lead to an increase in B-cell proliferation, antigen-specific immunoglobulin secretion, and IL-6 production [36,52]. Exposure of tonsillar B cells to CpG ODN induces a concentration- and time-dependent up-regulation of the activation markers CD23, CD25, CD40, CD54, CD80, CD86, and HLA-DR, and counteracts IgE production induced by IL-4 [53]. CpG directly induces T-bet expression and inhibits IgG1 and IgE switching in B cells, which is induced by IL-4 and CD40 signaling [54]. In murine models, pre-administration of CpG ODN in vivo prevented allergen-induced Th2 responses, including IL-5 production, eosinophilic airway inflammation, airway hyper-reactivity, and chronic airway remodeling [55–59].

CpG, or ISS-ODN, seems to stimulate the innate immune system to produce cytokines that drive the adaptive immune system toward a Th1-type immune

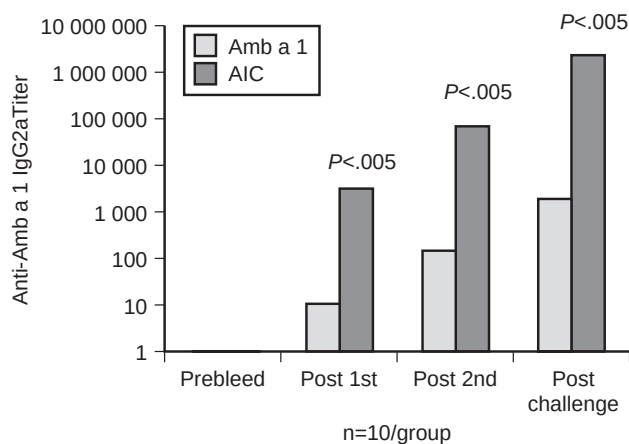


Fig. 3. IgG2a anti-Amb a 1 response in mice. Effect of immunization with AIC (solid bar) versus Amb a 1 alone (shaded bar) on IgG2a antibody production. Assays obtained pre-immunization, post-immunization (after the first and second vaccinations), and after challenge. Not shown are allergen mixed with ISS or allergen plus alum. (Adapted from Creticos PS. New methods in asthma and allergic rhinitis. *Advanced Studies in Medicine* 2002;2:273; with permission.)

AIC use results in the preferential induction of naïve CD4⁺ T cells, resulting in the differentiation toward a Th1 phenotypic profile. The corollary to this observation of a down-regulation in Th2-mediated responses to allergen (eg, IL-4, IL-5) is believed to be the result of dendritic cell (DC), macrophage or monocytic activation, and production of cytokines (IL-12, IL-18, IFN- α , IFN- β),

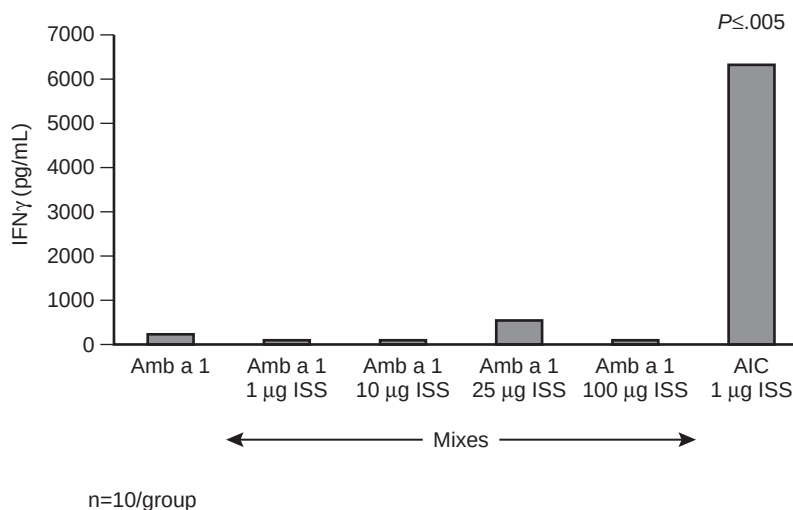


Fig. 4. In mice, effect of immunization on up-regulation of IFN- γ : AIC versus Amb a 1-ISS mixes versus Amb a 1 alone. (Adapted from Creticos PS. New methods in asthma and allergic rhinitis. *Advanced Studies in Medicine* 2002;2:273; with permission.)

with constituent effects on priming for Th1 development that results in increased IFN- γ on activation [8,9,32]. In a murine model, AIC immunization resulted in the specific up-regulation of IFN- γ (Th1 profile), which contrasts the Th2 cytokine dysregulation that otherwise would be observed from immunization with allergen alone, allergen plus alum, or allergen mixed with ISS but not linked to this specific adjuvant (Fig. 4). Marshall et al [9] observed similar results in in vitro studies of peripheral blood mononuclear cells that were obtained from human subjects with ragweed allergy. This research demonstrates that cells exposed to AIC produced significant levels of IFN- γ and had diminished production of IL-4 and IL-5.

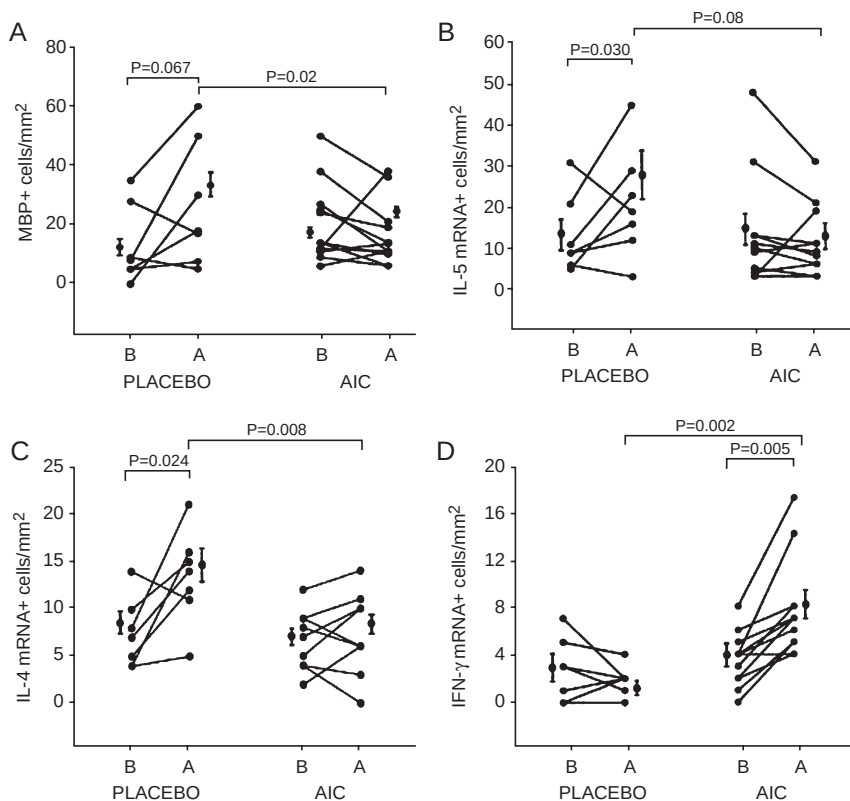


Fig. 5. Effect of AIC on allergen challenge before the ragweed season. The number of major basic protein (MBP)-positive cells (A), IL-5 mRNA-positive cells (B), IL-4 mRNA-positive cells (C), and IFN- γ mRNA-positive cells (D) at baseline (B) and after nasal allergen challenge with ragweed extract (A) in ragweed-sensitive patients 2 weeks after treatment with placebo ($n = 7$) or AIC ($n = 12$) is shown. Circles alongside the data represent mean values $\pm$ SEM. (Adapted from Tulic MK, Fisett PO, Christodoulou P, Vaillancourt P, Desrosiers M, Lavigne F, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol* 2004;113:239; with permission.)

Horner et al [60] showed that an optimal conjugation ratio of ISS-to-Amb a 1 resulted in an AIC that was approximately 100-fold less allergenic than native allergen, as measured by mast cell degranulation. Use of AIC also resulted in a marked reduction in anaphylactic death, and similar results were seen in a murine model of the Arthus reaction.

Clinical trials with Amb a 1 immunostimulatory oligonucleotide construct

Various clinical trials have been undertaken with the experimental AIC construct in France, Canada, and the United States. Data from the European studies have confirmed the safety of AIC when patients were immunized to 30 µg of Amb a 1 equivalent product [61]. The Canadian study by Hamid et al [62] demonstrated that AIC treatment resulted in a decrease in eosinophil migration and a shift from a Th2 profile to a Th1 profile (Fig. 5).

The authors' group at Johns Hopkins completed a Food and Drug Administration-defined clinical safety study in which quantitative intradermal end-point skin titration was employed to assess the relative potency of AIC to a standardized, commercially available ragweed preparation. The results demonstrated that the AIC product was more than 100-fold less reactive than the licensed ragweed extract when assessed with this parallel-line bioassay methodology. In a subset of patients, in vitro studies of basophil histamine release showed a similarly positive effect to that observed with the in vivo skin titration studies [64].

The authors' initial injection study demonstrated the tolerability and immunogenicity of AIC. Patients with ragweed allergy safely were dosed to 15 µg of AIC (based on Amb a 1 content) and demonstrated an immune response that was consistent with that for conventional ragweed immunotherapy [63].

The authors' clinical trial through the Immune Tolerance Network of the National Institutes of Health demonstrated encouraging clinical efficacy in patients with fall-seasonal allergic rhinitis. Patients experienced improvement in visual analog scores, seasonal symptom diary scores, quality-of-life scores, and medication use. These positive clinical observations were observed in the initial ragweed season, and improvement continued during the second season of observation, findings that provide evidence of sustained benefit [65].

Summary

The results from these clinical studies of patients with ragweed-induced allergic rhinitis demonstrated that AIC is less allergenic than conventional immunotherapeutic products and may offer the potential for an improved safety profile for immunotherapy. The initial phase I and phase II clinical trials with AIC demonstrated the improved immunogenicity and therapeutic potential of the construct and suggested that AIC may be a superior therapeutic agent, when compared with conventional immunotherapy. Further trials are underway to

evaluate and confirm the optimal dosing, long-term safety, immunologic effect, and therapeutic efficacy of AIC as a treatment modality for ragweed-induced allergic rhinitis and asthma.

References

- [1] Durham SR, Varney V, Gaga M, et al. Immunotherapy and allergic inflammation. *Clin Exp Allergy* 1991;21(Suppl 1):206–10.
- [2] Creticos PS. Immunotherapy with allergens. *JAMA* 1992;268:2834–9.
- [3] Creticos PS. Immunotherapy. In: Rich RR, Shearer WT, editors. *Clinical immunology, principles and practice*. St. Louis (MO): Mosby; 1995. p. 2002–18.
- [4] Creticos PS, Reed CE, Norman PS, et al. Ragweed immunotherapy in adult asthma. *N Engl J Med* 1996;334:501–6.
- [5] Creticos PS. The consideration of immunotherapy in the treatment of allergic asthma. *J Allergy Clin Immunol* 2000;105:S559–74.
- [6] Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999;341:468–75.
- [7] Bousquet J, Lockey R, Malling HJ, et al. Allergen immunotherapy: therapeutic vaccines for allergic diseases: World Health Organization, American Academy of Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol* 1998;81:401–5.
- [8] 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* 2000;106:124–34.
- [9] Marshall JD, Abtahi S, Eiden JJ, et al. Immunostimulatory sequence DNA linked to the Amb a 1 allergen promotes T(H)1 cytokine expression while downregulating T(H)2 cytokine expression in PBMCs from human patients with ragweed allergy. *J Allergy Clin Immunol* 2001;108:191–7.
- [10] Ishizaka T, Hirata F, Ishizaka K, et al. Stimulation of phospholipid methylation. Ca²⁺ + influx, and histamine release by bridging of IgE receptors on rat mast cells. *Proc Natl Acad Sci U S A* 1980;77:1903–6.
- [11] Wasserman SI. Mediators of immediate hypersensitivity. *J Allergy Clin Immunol* 1983;372:101–15.
- [12] Lewis RA, Austen KF. Mediation of local homeostasis and inflammation by leukotrienes and other mast cell dependent compounds. *Nature* 1981;293:103–8.
- [13] Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol* 2003;111:450–63.
- [14] O’Hehir RE, Garman RD, Greenstein JL, Lamb JR. The specificity and regulation of T-cell responsiveness to allergens. *Annu Rev Immunol* 1991;9:67–95.
- [15] Robinson DS, Hamid Q, Jacobson M, et al. Evidence for Th2-type T helper cell control of allergic disease in vivo. *Springer Semin Immunopathol* 1993;15:17–27.
- [16] Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA + cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997;99:254–60.
- [17] Nakamura Y, Ghaffar O, Olivenstein R, Taha RA, Soussi-Gounni A, Zhang DH, et al. Gene expression of the GATA-3 transcription factor is increased in atopic asthma. *J Allergy Clin Immunol* 1999;103:215–22.
- [18] Finotto S, Neurath MF, Glickman JN, Qin S, Lehr HA, Green FH, et al. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science* 2002;295:336–8.
- [19] Creticos PS, Van Metre TE, Mardiney MR, et al. Dose response of IgE and IgG antibodies during ragweed immunotherapy. *J Allergy Clin Immunol* 1984;73:94–104.
- [20] Creticos PS. Immunotherapy with allergens. *JAMA* 1997;268:2834–9.

- [21] Lichtenstein LM, Ishizaka K, Norman PS, Hill BM. IgE antibody measurements in ragweed hay fever: relationship to clinical severity and the results of immunotherapy. *J Clin Invest* 1973; 52:472–82.
- [22] Haugaard L, Dahl R, Jacobsen L. A controlled dose-response study of immunotherapy with standardized, partially purified extract of house dust mite: clinical efficacy and side effects. *J Allergy Clin Immunol* 1993;91:709–22.
- [23] Creticos PS, Adkinson Jr NF, Kagey-Sobotka A, et al. Nasal challenge with ragweed pollen in hay fever patients: effect of immunotherapy. *J Clin Invest* 1985;76:2247–53.
- [24] Creticos PS, Marsh DG, Proud D, et al. Responses to ragweed-pollen nasal challenge before and after immunotherapy. *J Allergy Clin Immunol* 1989;84:197–205.
- [25] Iliopoulos O, Proud D, Adkinson Jr NF, et al. Effects of immunotherapy on the early, late, and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 1991;87:855–66.
- [26] Furin MJ, Norman PS, Creticos PS, et al. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. *J Allergy Clin Immunol* 1991;88:27–32.
- [27] Varney VA, Hamid QA, Gaga M, 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.
- [28] Durham SR, Ying S, Varney VA, 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-gamma. *J Allergy Clin Immunol* 1996;97: 1356–65.
- [29] Ebner C, Siemann U, Bohle B, et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin Exp Allergy* 1997;27:1007–15.
- [30] Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998;102:157–64.
- [31] 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.
- [32] Tighe H, Takabayashi K, Schwartz D, et al. Conjugation of protein to immunostimulatory DNA results in a rapid, long-lasting and potent induction of cell-mediated and humoral immunity. *Eur J Immunol* 2000;30:1939–47.
- [33] Tokunaga T, Yamamoto H, Shimada S, et al. Antitumor activity of deoxyribonucleic acid fraction from *Mycobacterium bovis* BCG: I. Isolation, physicochemical characterization, and antitumor activity. *J Natl Cancer Inst* 1984;72:955–62.
- [34] Yamamoto S, Yamamoto T, Shimada S, et al. DNA from bacteria, but not from vertebrates, induces interferons, activates natural killer cells and inhibits tumor growth. *Microbiol Immunol* 1992;36:983–97.
- [35] Yamamoto T, Yamamoto S, Kataoka T, Tokunaga T. Ability of oligonucleotides with certain palindromes to induce interferon production and augment natural killer cell activity is associated with their base length. *Antisense Res Dev* 1994;4:119–22.
- [36] Krieg AM, Yi AK, Matson S, et al. CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* 1995;374:546–9.
- [37] Bird AP. DNA methylation and the frequency of CpG in animal DNA. *Nucleic Acids Res* 1980; 8:1499–504.
- [38] Kuramoto E, Yano O, Kimura Y, et al. Oligonucleotide sequences required for natural killer cell activation. *Jpn J Cancer Res* 1992;83:1128–31.
- [39] Krieg AM. CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol* 2002;20:709–60.
- [40] Krug A, Rothenfusser S, Hornung V, et al. Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. *Eur J Immunol* 2001; 31:2154–63.

- [41] Duramad O, Fearon KL, Chan JH, Kanzler H, Marshall JD, Coffman RL, et al. IL-10 regulates plasmacytoid dendritic cell response to CpG-containing immunostimulatory sequences. *Blood* 2003;102:4487–92.
- [42] Marshall JD, Hessel EM, Gregorio J, Abbate C, Yee P, Chu M, et al. Novel chimeric immunomodulatory compounds containing short CpG oligodeoxyribonucleotides have differential activities in human cells. *Nucleic Acids Res* 2003;31:5122–33.
- [43] Hemmi H, Takeuchi O, Kawai T, et al. A Toll-like receptor recognizes bacterial DNA. *Nature* 2000;408:740–5.
- [44] Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, Knetter CF, et al. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat Immunol* 2004;5:190–8.
- [45] Sabroe I, Parker LC, Wilson AG, Whyte MKB, Dower SK. Toll-like receptors: their role in allergy and non-allergic inflammatory disease. *Clin Exp Allergy* 2002;32:984–9.
- [46] Hornung V, Rothenfusser S, Britsch S, et al. Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J Immunol* 2002;168:4531–7.
- [47] Kadowaki N, Ho S, Antonenko S, et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* 2001;194:863–9.
- [48] Rothenfusser S, Hornung V, Krug A, et al. Distinct CpG oligonucleotide sequences activate human gamma delta T cells via interferon-alpha/-beta. *Eur J Immunol* 2001;31:3525–34.
- [49] Bauer M, Redecke V, Ellwart JW, et al. Bacterial CpG-DNA triggers activation and maturation of human CD11c-, CD123 + dendritic cells. *J Immunol* 2001;166:5000–7.
- [50] Rothenfusser S, Tuma E, Endres S, Hartmann G. Plasmacytoid dendritic cells: the key to CpG(1). *Hum Immunol* 2002;63:1111–9.
- [51] Krug A, Towarowski A, Britsch S, et al. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur J Immunol* 2001;31:3026–37.
- [52] Yi AK, Klinman DM, Martin TL, Matson S, Krieg AM. Rapid immune activation by CpG motifs in bacterial DNA: systemic induction of IL-6 transcription through an antioxidant-sensitive pathway. *J Immunol* 1996;157:5394–402.
- [53] Gantner F, Hermann P, Nakashima K, et al. CD40-dependent and -independent activation of human tonsil B cells by CpG oligodeoxynucleotides. *Eur J Immunol* 2003;33:1576–85.
- [54] Liu N, Ohnishi N, Ni L, Akira S, Bacon KB. CpG directly induces T-bet expression and inhibits Ig(gamma1) and Ig(epsilon) switching in B cells. *Nat Immunol* 2003;4:687–93.
- [55] Broide D, Schwarze J, Tighe H, et al. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J Immunol* 1998;161:7054–62.
- [56] Broide DH, Stachnick G, Castaneda D, et al. Systemic administration of immunostimulatory DNA sequences mediates reversible inhibition of Th2 responses in a mouse model of asthma. *J Clin Immunol* 2001;21:175–82.
- [57] Kline JN, Waldschmidt TJ, Businga TR, et al. Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. *J Immunol* 1998;160:2555–9.
- [58] Sur S, Wild JS, Choudhury BK, et al. Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligodeoxynucleotides. *J Immunol* 1999;162:6284–93.
- [59] Jain VV, Kitagaki K, Businga T, et al. CpG-oligodeoxynucleotides inhibit airway remodeling in a murine model of chronic asthma. *J Allergy Clin Immunol* 2002;110:867–72.
- [60] Horner AA, Takabayashi K, Beck L, et al. Optimized conjugation ratios lead to allergen immunostimulatory oligodeoxynucleotide conjugates with retained immunogenicity and minimal anaphylactogenicity. *J Allergy Clin Immunol* 2002;110:413–20.
- [61] Dieudonné F, Vital Durand D, Eiden J, Tuck G, Van Nest G, Raz E, Hamilton R, et al. ISS linked to Amb a 1 allergen (AIC) stimulates IgG response to Amb a 1 by ragweed-allergic humans [abstract]. *J Allergy Clin Immunol* 2001;107:933.

- [62] Tulic MK, Fiset PO, Christodouloupoulos P, Vaillancourt P, Desrosiers M, Lavigne F, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol* 2004;113:235–41.
- [63] Creticos PS, Eiden JJ, Balcer SL, Van Nest G, Kagey-Sobotka A, Tuck SF, et al. Immunostimulatory oligonucleotides conjugated to Amb a 1: safety, skin test reactivity, and basophil histamine release [abstract]. *J Allergy Clin Immunol* 2000;105:215.
- [64] Creticos PS, Balcer SL, Schroeder JT, Hamilton RG, Chung B, Norman P, et al. Initial immunotherapy trial to explore the safety, tolerability and immunogenicity of subcutaneous injections of an Amb a 1 immunostimulatory oligonucleotide conjugate [AAIC] in ragweed allergic adults [abstract]. *J Allergy Clin Immunol* 2001;107(Part 2):S216.
- [65] Creticos PS, Eiden JJ, Broide DH, Balcer-Whaley SL, Schroeder JT, Khattignavong A, 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 [abstract]. *J Allergy Clin Immunol* 2002;109:743–4.