

Immunological approaches to the treatment of food allergy

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Introduction

Food allergic reactions have engendered increasing concern in the United States, with about one-quarter of American households altering their dietary habits because a member of the family is perceived to suffer from food allergies (1). Prospective studies have indicated that about 5% of children less than 4 years of age experience IgE-mediated food allergic reactions, with about 2.5% of young children reacting to cow's milk, about 1.3% to hen's egg and 0.5% to peanut (2). Children with atopic disorders, especially atopic dermatitis, are more often affected by food allergies. About 35% of children with moderate to severe atopic dermatitis have skin symptoms provoked by food hypersensitivity (3). The prevalence of food hypersensitivity in adults is reportedly less common, but a recent survey in the United States found that 1.3% of adults are allergic to peanuts or tree nuts (4). Given the estimated frequency of allergy to fish, shellfish and other sensitivities, it is likely that about 2% of the adult population, or about 5.5 million Americans, are affected by food allergies (2).

In spite of increased recognition and understanding about food allergies, they are the single most common cause of generalised anaphylaxis seen in hospital emergency departments (5), accounting for about one-third of anaphylaxis cases seen. It is estimated that about 125 – 150 fatal cases of food-induced anaphylaxis occur in

the United States each year (6, 7). Pumphrey in the United Kingdom (8) and Moneret-Vautrin in France (9) reported similar findings. A recent survey of South Australian preschool and school-age children revealed a parent-reported food anaphylaxis rate of 0.43 per 100 school children, which accounted for over one-half of all cases of anaphylaxis in this age group (10). Similarly, Novembre reported that food allergy was responsible for about one-half of severe anaphylactic episodes in Italian children treated in emergency departments (11). While food anaphylaxis accounts for one-third to one-half of anaphylaxis cases treated in emergency departments in North America, Europe and Australia (7, 8, 11–13), it is uncommon in most Asian countries.

At the present time, strict avoidance of food allergens and ready access to self-injectable epinephrine is the "standard of care" for food allergy (14). Earlier reports of "desensitising" patients with subcutaneous injections of food extracts were poorly controlled and unconvincing (15). Similarly, early reports of oral desensitisation were not well controlled. Recently Patriarca et al. reported that 12 food-allergic children were successfully "desensitised" to several foods utilising an oral desensitisation protocol, but no post-desensitisation food challenges were performed to confirm loss of reactivity (16). In 1997, Nelson and coworkers published their results with a trial of subcuta-

neous "rush" immunotherapy in peanut-allergic patients (17). In this well-controlled, double-blind placebo-controlled trial of peanut immunotherapy, subjects underwent double-blind placebo-controlled food challenge (DBPCFCs) prior to and following the "desensitisation" protocol. While four of six treated patients demonstrated increased tolerance to peanut on the second challenge (no change in the placebo group), the maintenance dose was decreased in two cases (with concomitant decrease in tolerance) because of frequent systemic reactions. Given the partial rate of response and high rate of adverse reactions to traditional immunotherapy, alternative forms of immunotherapy are needed for this potentially fatal allergy.

Several novel immunotherapeutic strategies are being examined as treatment modalities for food allergy: 1) humanised anti-IgE monoclonal antibody therapy 2), "engineered" (mutated) allergen protein immunotherapy 3), antigen-immunostimulatory sequence-modulated immunotherapy 4), peptide immunotherapy 5), plasmid-DNA immunotherapy and 6) cytokine-modulated immunotherapy. The status of the first four approaches will be reviewed.

In atopic and food-allergic patients, the generation of allergen-specific IgE antibodies that bind to high affinity receptors [Fc ϵ RI] on the surface of mast cells and basophils is central to the immunopathology of these disorders. *Humanised, monoclonal anti-IgE antibodies* have been developed that bind to the third domain of the Fc region of IgE molecules, thus preventing their binding to Fc ϵ RI or Fc ϵ RII. A number of clinical trials utilising these antibodies have reported symptomatic improvement in patients with allergic asthma and rhinitis when circulating levels of IgE antibodies are markedly reduced (18–22). In addition, it has been found that basophil histamine releasability and Fc ϵ RI expression are downregulated during anti-IgE therapy (23). In conjunction with five other centers in the United States, we have initiated a double-blind placebo-controlled trial of Tanox's humanised, monoclonal anti-IgE antibody in the treatment of patients with peanut-induced anaphylaxis. To date, 28 patients have been enrolled in this double-blind trial. In this study, the quantity of peanut necessary to provoke an allergic reaction is established by DBPCFC. Patients then receive four subcutaneous injections of anti-IgE or placebo at monthly intervals and then undergo a second peanut challenge. If successful, this form of therapy could provide food-allergic patients with relief from virtually any food allergen,

although continued protection will be dependent upon indefinite monthly injections.

Unlike anti-IgE therapy that may prove therapeutic to a wide variety of allergens, other immunotherapeutic strategies are directed at specific allergens. Since IgE-mediated allergic diseases are the result of an overproduction of Th2 cytokines by allergen-specific CD4 $^{+}$ T cells, several investigators have attempted to use other approaches to redirect allergen-specific Th2 responses to Th1 responses.

In collaboration with Drs Burks and Bannon, we have developed *engineered major allergenic proteins* to eliminate IgE binding but retain T cell proliferation. We have isolated and purified the three major peanut proteins (Ara h1, Ara h2, and Ara h3); isolated, sequenced and cloned the DNA that codes for the synthesis of these proteins; and mapped the IgE-binding epitopes. With this information, these proteins have been "engineered" to eliminate the IgE-binding epitopes. Using site-directed mutagenesis of the allergen cDNA clones followed by recombinant production of the modified allergen, the engineered DNA codes for proteins that differ by a single amino acid within each of the IgE-binding epitopes. Ara h1 is a vicilin storage protein that is 626 amino acids in length and has 23 IgE-binding sites (epitopes). Ara h2 is a conglutin storage protein that is 156 amino acids in length and has 10 IgE-binding sites and Ara h 3 is a glycinin storage protein that is 510 amino acids in length and has 4 IgE-binding sites. The engineered recombinant proteins bind virtually no IgE antibodies from peanut-allergic patients but promote comparable T cell proliferation as the native peanut proteins. We hypothesised that these engineered proteins will not provoke any allergic reaction when injected into peanut sensitised mice, but that they will be able to downregulate the allergic response. As suggested by *in vitro* studies with Bet v1 and Phl p 5 (24, 25), studies in our peanut-anaphylaxis mouse model (26) suggested that this is true. Following our standard 3-week sensitisation protocol with Ara h2 and cholera toxin, mice received 3 doses/week of either native or engineered Ara h2 for 4 weeks (Fig. 1). Ara h2-specific IgE levels were decreased in the engineered Ara h2-treated group and increased in the native Ara h2-treated group compared to the PBS-treated controls. While the native Ara h2-treated group experienced decreased symptoms compared to the controls when challenged at week 8, the engineered Ara h2-treated group had significantly less symptoms than the native Ara h2 group (Fig. 2). While this approach seems very promising, there are a few

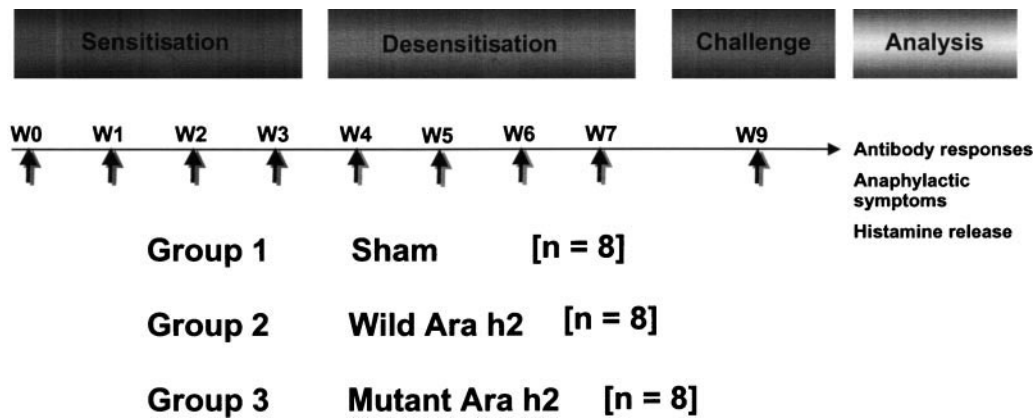


Fig. 1. Sensitisation and challenge with “engineered” Ara h2 protocol.

drawbacks with respect to this strategy when looking ahead to the treatment of other food allergies. This approach requires the need to identify IgE-binding sites for all major allergenic proteins in each food that is responsible for the allergic reaction (e.g. 4–5 proteins in milk, 3 proteins in egg, etc.). It also necessitates the need to isolate and “engineer” the DNA encoding each of the allergenic proteins, and it will require considerable effort to produce sufficient quantities of each engineered food protein for use in human trials.

Oligonucleotides immunostimulatory sequences (ISS-ODN) containing unmethylated palindromic CpG motifs have been shown to possess immunomodulatory properties that decrease airway hyperreactivity, lung eosinophilia and allergen-specific IgE production when administered during allergen sensitisation (27–29). These nucleotide sequences activate antigen-presenting cells (primarily monocytes) to secrete IFN- α , β , IL-6, IL-12 and IL-18, and NK cells to secrete IFN- γ , both of which promote Th1 responses. In collaboration with Dynavax Inc. (Berkley, CA, USA) we are investigating the use of CpG-conjugated-Ara h2 in our mouse model of peanut-induced anaphylaxis. The use of ISS-ODNs as adjuvants with native proteins was found to be an effective form of immunotherapy to prevent IgE-mediated peanut allergy (Fig. 3). We are now conducting trials to determine whether ISS-Ara h2 is equally effective in reversing established peanut allergy in our mouse model. Other adjuvants that induce deviation of antigen-specific Th2 responses to Th1 responses also could be utilised and include antigen-linked cytokines (e.g. IL-12, IL-18) (30) or heat-killed *Listeria monocytogenes* (31). One potentially promising method being investigated is to use *Listeria* as the expression system for the modified peanut proteins. After the bacteria is

transfected with the peanut protein plasmid-DNA and expresses the protein in the cytoplasm, it is heat-killed and then injected into the subject where it would be taken up rapidly by the APCs and promote an antigen-specific Th1 response.

Another approach being investigated is the use of overlapping peptides. Vaccines comprised of small peptides (10–20-mers) that represent the entire length of a specific food protein are generated. For example, with Ara h2 (a major peanut protein), we have generated thirty 20-mers that overlap by 15 amino acids:

Ara h 2=156 amino acids in length –
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
 24 25
 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
 27 28 29 30

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137 138 139 140 141 142 143 144 145 146
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In this way, APCs are provided with all the possible T cell binding epitopes. Peptides can be

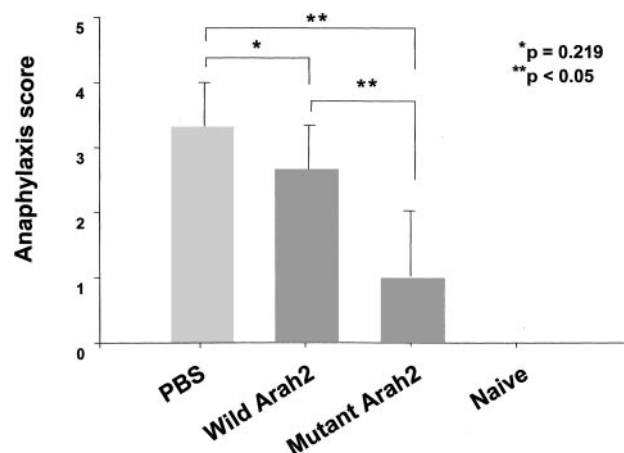


Fig. 2. Symptom scores following Ara h2 intragastric challenge of mice treated with native and “engineered” Ara h2 protocol.

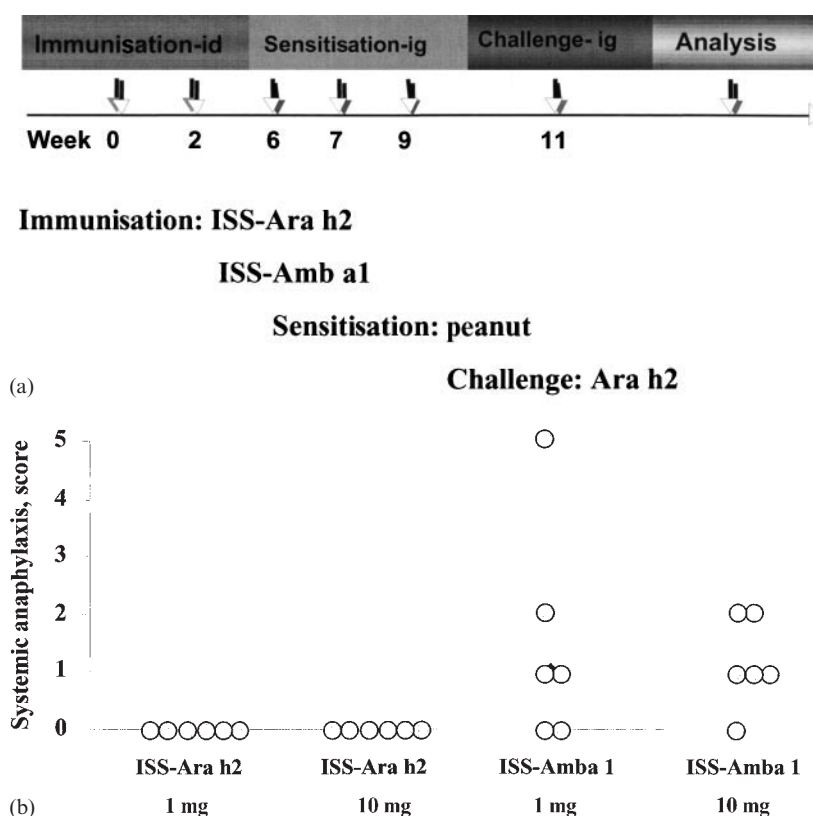


Fig. 3. (a) Experimental protocol for prevention of Ara h2 hypersensitivity with ISS-conjugated Ara h2. (b) Symptom scores following Ara h2 intragastric challenge of mice pretreated with ISS-conjugated Ara h2 and irrelevant antigen, Amb a1.

screened to determine whether IgE antibodies from peanut-allergic patients bind to any of these peptide fragments. Peptides that bind IgE are discarded. With the extensive overlap of the peptides, ample T-cell epitopes should be retained even if we discard a number of the peptides. By eliminating IgE-binding peptides, the peptide vaccine will not provoke an allergic reaction but will still “downregulate” the allergic response. Preliminary studies in our anaphylactic mouse model with 20-mers suggest that this approach will be very effective. C3H/HeJ mice

were sensitised to Ara h2. Desensitisation was initiated 2 weeks later with intranasal or subcutaneous Ara h2 peptide mix (low dose: 2 µg or high dose: 20 µg/mouse) for 3 consecutive days a week for 4 weeks. Mice were then challenged with intragastric Ara h2. The high-dose intranasal and subcutaneous desensitisation regimens led to significantly decreased symptom scores and plasma histamine levels compared to sham-treated mice (Fig. 4). Ara h2-specific serum IgE levels remained significantly lower than sham-treated mice following the first and second oral

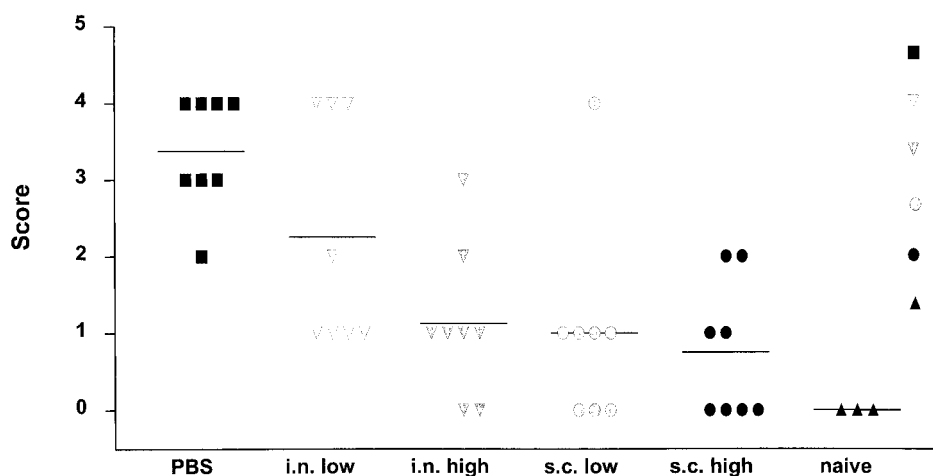


Fig. 4. Symptom scores following Ara h2 intragastric challenge of sensitised mice treated with intranasal or subcutaneous peptide immunotherapy.

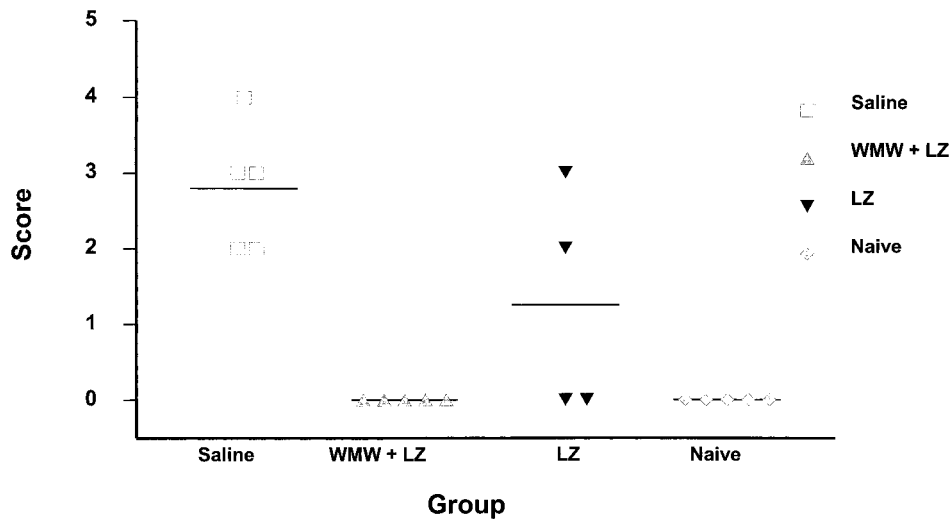


Fig. 5. Symptom scores following Ara h2 intragastric challenge of peanut-sensitised mice treated with traditional Chinese medicine.

challenge. Ara h2-stimulated spleen cells *in vitro* synthesised similar quantities of IL-4 and IL-5 compared to sham-treated mice, but IFN- γ production in peptide-treated mice spleen cells was markedly increased compared to sham-treated mice. Studies are under way to determine the optimal dose of peptide mix for desensitisation of peanut anaphylaxis.

We are now generating overlapping peptides for Ara h1 and Ara h3, removing IgE-binding peptides, and developing a vaccine with all three peanut protein peptide mixtures to determine if mice sensitised to whole peanut can also be desensitised. If this approach is successful, there are several distinct advantages: IgE binding sites for each food protein do not need to be mapped, allergen-specific DNA does not need to be mutated, engineered recombinant proteins do not need to be produced. With this approach, vaccines for any food in which the responsible allergenic proteins are known can be formulated. Kay and Larche demonstrated recently that the intradermal injection of overlapping peptides from chain 1 of the cat allergen, Fel d1, did not cross-link IgE but did elicit an isolated late asthmatic response with no visible early response in 9/40 cat-allergic asthmatics (32). They have gone on to demonstrate that repeated injections lead to suppression of both the early and late-phase reaction, thus providing a potentially effective form of immunotherapy (personal communication).

A number of exciting new immunotherapeutic strategies are being evaluated by a number of investigators. In addition we have been evaluating the potential role of traditional Chinese medicines in the modification of food-induced

anaphylactic responses. We recently reported on the effectiveness of one herbal remedy, MSSM-002, for treating airway reactivity (33). Although food-induced anaphylactic reactions were not described in the traditional Chinese literature, Dr Xiumin Li in our group has developed a formulation (WMW-LZ) based on a number of traditional gastrointestinal remedies that was found to reduce serum IgE and completely suppress the anaphylactic response to peanut ingestion in peanut-sensitive mice (Fig. 5). With the increasing interest in this area, effective forms of therapy for IgE-mediated food allergies should be available in the next several years.

References

1. SLOAN AE, POWERS ME. A perspective on popular perceptions of adverse reactions to foods. *J Allergy Clin Immunol* 1986; 78: 127-33.
2. SAMPSON HA. Food allergy. Part 1: Immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999; 103: 717-28.
3. EIGENMANN PA, SICHERER SH, BORKOWSKI TA, COHEN BD, SAMPSON HA. Prevalence of IgE-mediated food allergy among children with atopic dermatitis. *Pediatrics* 1998; 101: e8.
4. SICHERER SH, MUNOZ-FURLONG A, BURKS AW, SAMPSON HA. Prevalence of peanut and tree nut allergy in the United States of America. *J Allergy Clin Immunol* 1999; 103: 559-62.
5. YOCUM MW, KHAN DA. Assessment of patients who have experienced anaphylaxis: a 3-year survey. *Mayo Clin Proc* 1994; 69: 16-23.
6. SAMPSON HA. Fatal food-induced anaphylaxis. *Allergy* 1998; 53: 125-30.
7. YOCUM MW, BUTTERFIELD JH, KLEIN JS, VOLCHECK GW, SCHROEDER DR, SILVERSTEIN MD. Epidemiology of anaphylaxis in Olmsted County: a population-based study. *J Allergy Clin Immunol* 1999; 104: 452-6.

8. PUMPHREY RSH, STANWORTH SJ. The clinical spectrum of anaphylaxis in north-west England. *Clin Exp Allergy* 1996; 26: 1364–70.
9. MONERET-VAUTRIN DA, KANNY G. Food-induced anaphylaxis. A new French multicenter survey. *Ann Gastroenterol Hepatol* (Paris) 1995; 31: 256–63.
10. BOROS CA, KAY D, GOLD MS. Parent reported allergy and anaphylaxis in 4173 south Australian children. *J Paediatr Child Health* 2000; 36: 36–40.
11. NOVEMBRE E, CIANFERONI A, BERNARDINI R, et al. Anaphylaxis in children: clinical and allergologic features. *Pediatrics* 1998; 101: e8–1–e8/8.
12. KEMP SF, LOCKEY RF, WOLF BL, LIEBERMAN P. Anaphylaxis: a review of 266 cases. *Arch Intern Med* 1995; 155: 1749–54.
13. LEE JM, GREENES DS. Biphasic anaphylactic reactions in pediatrics. *Pediatrics* 2000; 106: 762–6.
14. SAMPSON HA. Food Allergy. Part 2: Diagnosis and management. *J Allergy Clin Immunol* 1999; 103: 981–99.
15. GOLDSTEIN GB, HEINER DC. Clinical and immunological perspectives in food sensitivity. *J Allergy* 1970; 46: 270–91.
16. PATRIARCA G, SCHIAVINO D, NUCERA E, SCHINCO G, MILANI A, GASBARRINI GB. Food allergy in children: results of a standardized protocol for oral desensitization. *Hepato-gastroenterology* 1998; 45: 52–8.
17. NELSON HS, LAHR J, RULE R, BOCK SA, LEUNG DYM. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J Allergy Clin Immunol* 1997; 99: 744–51.
18. FAHY JV, FLEMING E, WONG HH, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1997; 155: 1828–34.
19. BOULET LP, CHAPMAN KR, COTE J, et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response 1997; 155: 1835–40.
20. DEMOLY P, BOUSQUET J. Anti-IgE therapy for asthma. *Am J Resp Crit Care Med* 1997; 155: 1825–7.
21. MILGROM H, FICK RB, SU JQ, et al. Treatment of allergic asthma with monoclonal anti-IgE antibody. *N Engl J Med* 1999; 341: 1968–73.
22. CASALE TB, BERNSTEIN IL, BUSSE W, et al. Use of anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. *J Allergy Clin Immunol* 1997; 100: 100–10.
23. MACGLASHAN DWJ, BOCHNER BS, ADELMAN DC, et al. Down-regulation of Fc (epsilon)RI expression on human basophils during *in vivo* treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997; 158: 1438–45.
24. FERREIRA F, EBNER C, KRAMER B, et al. Modulation of IgE reactivity of allergens by site-directed mutagenesis: potential use of hypoallergenic variants for immunotherapy. *FASEB* 1998; 12: 231–42.
25. SCHRAMM G, KAHLERT H, SUCK R, et al. “Allergen engineering”: variants of the timothy grass pollen allergen Phl p 5b with reduced IgE-binding capacity but conserved T cell reactivity. *J Immunol* 1999; 162: 2406–14.
26. LI XM, SEREBRISKY D, LEE SY, et al. A murine model of IgE-mediated peanut anaphylaxis. *J Allergy Clin Immunol* 2000; 106: 150–8.
27. 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.
28. KLINE JN, WALDSCHMIDT TJ, BUSINGA TR, et al. Modulation of airway inflammation by CpG oligonucleotides in a murine model of asthma. *J Immunol* 1998; 160: 2555–9.
29. KLINE JN, KRIEG AM, WALDSCHMIDT TJ, BALAS JK, 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.
30. KIM TS, DEKRUYFF RH, RUPPER R, MAECKER HT, LEVY S, UMETSU DT. An ovalbumin-IL-12 fusion protein is more effective than ovalbumin plus free recombinant IL-12 in inducing a T helper cell type-dominated immune response and inhibiting antigen-specific IgE production. *J Immunol* 1997; 158: 4137–44.
31. YEUNG VP, GIENI RS, UMETSU DT, DEKRUYFF RH. Heat-killed *Listeria monocytogenes* as an adjuvant converts established murine Th-2-dominated immune responses into Th1-dominated responses. *J Immunol* 1998; 161: 4146–52.
32. HASELDEN BM, KAY AB, LARCHE M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999; 189: 1885–94.
33. LI XM, HUANG CK, ZHANG TF, et al. The Chinese herbal medicine formula, MSSM-002, suppresses allergic airway hyperreactivity and modulates Th1/Th2 responses in a murine model of allergic asthma. *J Allergy Clin Immunol* 2000; 106: 660–8.