

EDITORIAL

Hunting the magic bullet in immunotherapy: new forms of old treatment or something completely different?

Specific immunotherapy (SIT) has been shown to be a highly effective mode of treatment in allergic rhinoconjunctivitis [1] and is the preferred method of management of severe hymenoptera venom allergy [2]. Asthma with a strong allergic component is also seen as an indication for immunotherapy in many countries but controversy still exists [3]. The basic principles of SIT have not really changed since the first observations of Noon and Freeman published on 'innoculation' treatment for hay fever [4]. In the current issue of this journal, Leynadier et al. report how the use of calcium phosphate as an adjuvant may have future therapeutic potential [5]. They have shown it to have immunologically beneficial activity and a similar degree of efficacy to aluminium hydroxide bound grass pollen mixture, which is conventionally used as adjuvant. One particular advantage is that the subcutaneous nodules and IgE responses seen with conventional SIT preparations appear to be avoided. Nevertheless, further direct comparison between this alternative adjuvant and conventional treatment is needed for it to become widely accepted.

At present the WHO [6] and EAACI [7] position papers outline guidelines for current practice of immunotherapy and possible future directions. There are now several large randomized control trials that illustrate the efficacy of grass pollens [8], house dust mite [9] and cat dander [10] immunotherapy for allergic rhinoconjunctivitis. Other studies have shown that in sensitized children, asthma may be prevented with immunotherapy [11]. A reduction in symptoms and rescue medication and an objectively improved quality of life with grass pollen SIT is now clearly established, whilst the benefit gained from both grass pollen and venom immunotherapy persists long after therapy ends [12]. Conventional practice requires standardized vaccines of known stability and shelf life but less commonly used vaccines may not be as well standardized [13]. The mechanism by which SIT exerts a therapeutic effect remains to be fully described and several mechanisms may be responsible. T cell response – either anergy of Th2 cells or deviation to a Th1 cell cytokine profile – is the key part of the observed clinical outcome and has been demonstrated *in vitro* [14]. Specific IgG₄ increases with SIT, a mechanism which was previously viewed as the prime candidate for the mechanism of immunotherapy [15].

Safety and the side-effect profile of SIT is well described

and anaphylaxis occurs in a recognized percentage of patients [16], although more vigilant practice and improved standardization of vaccines has resulted in fewer severe outcomes. There appears to be no difference in severe reaction risk between different allergens. There are drawbacks to conventional SIT, primarily because it is time-consuming, severe anaphylactic reactions may occur and preparations are combinations of molecules of which only some are immunoactive. Alternative dosages, regimes and duration of treatment are well documented elsewhere [17–19]. More recently, nasal, bronchial [20], oral [21] and sublingual [22] routes of delivery have been investigated as possible alternatives to the usual subcutaneous route. However, modifying allergen to tailor the target cell response offers the most intriguing prospects for the future of SIT. By whatever means they can be achieved, improved safety and efficacy would increase patient compliance and satisfaction and ultimately lead to increased use of immunotherapy by physicians.

Recombinant allergens

Recombinant technology allows the selection of less allergenic isoforms, expression of larger polypeptides or smaller fragments and also site-directed mutagenesis to alter the conformation of epitopes [23]. Reduction in IgE binding capacity by modification of B-cell epitopes without loss of T cell epitopes is possible and promises increased efficacy and safety, by allowing T cell interaction whilst avoiding the risk of IgE cross-linking and mast cell/basophil degranulation. Alteration of the three-dimensional structure of an antigen will influence the type of cell employed to present antigen [24]. Antigen-presenting cells (APCs) fulfil the tasks of capturing and processing the antigen before presentation to the T cell by MHC molecules on the cell surface. B cells that present bee venom phospholipase A (PLA) antigen do not secrete pro Th1 costimulatory molecules as released by monocytes or macrophages. Thus Th2 cytokines and IgE production are not suppressed. Conversely if monocytes present antigen there is increased T cell proliferation and decreased IL-4, IL-5 and IL-13 as well as reduced IgE production.

A number of *in vitro* studies have shown that modified Fel d 1 [25] and Lep d 2 [26] do not stimulate specific

antibody activity but are internalized by macrophages and monocytes, replicating the findings with PLA antigen. Recombinant technology can produce different isoforms which offer a spectrum of reactivity. Very reactive forms are likely to find a role in diagnosing allergy and forms that are unable to produce an IgE response will be applied in immunotherapy. A single amino acid variation is able to produce changes in T cell reactivity. The principal allergen of birch pollen, Bet v1, has been cloned and modified to produce several fragments which have T cell activity, demonstrating potential clinical efficacy [27]. The resulting disruption of three-dimensional structure yields molecules that do not cause histamine release and clinically produces a smaller skin test reaction in birch pollen-allergic individuals.

Site-directed mutagenesis aims, with knowledge of the genetic sequence of a protein, to decrease antibody binding and therefore IgE-mediated immediate hypersensitivity reactions. The allergen Der p 2 has been modified using this technique so that disruption of disulphide bonds reduces IgE binding without loss of T cell reactivity [28]. Tertiary structure has been predicted for this molecule and surface amino acid sequence altered such that skin testing elicits a response 1% of that of unmodified allergen.

Chemically modified allergens (allergoids)

Potassium cyanate, formaldehyde and glutaraldehyde have been used to chemically modify allergen to reduce allergenicity, as measured by skin tests and *in vitro* histamine release, without loss of T cell immunogenicity. Glutaraldehyde is known to act as a cross-linking agent, producing large polymeric molecules. Potassium cyanate substitutes lysine residues and produces a beneficial effect by impairing IgE binding. The value of chemical modification is controversial, with some authors reporting little clinical benefit when comparing native allergen with allergen modified by heat, formalin and acetylation [29], whereas others have found good clinical benefit and a very good IgG response in allergic and non-allergic individuals treated with allergoids [30,31].

Adjuvants

The purpose of an adjuvant is to improve the response to therapy by altering the physical properties of allergen. Previously alum, mineral oil, urea denaturation, incomplete Freund's adjuvant and tyrosine adsorbate have been used to do this, primarily by slowing absorption from injection sites. Alum slows absorption of conjugated allergen and this decreases the number of injections needed to elicit a similar immunological response; tyrosine works in a similar fashion. Complete Freund's adjuvant has been

shown to induce macrophage IL-12 production and a predominantly Th1 cytokine profile [32]. In SIT trials alum has been shown to elevate 'blocking' IgG and produce better clinical outcomes than unconjugated vaccines [33]. Polymerization of allergen into high molecular weight polymers is known to offer improved efficacy over unmodified allergen, possibly by impairing binding to B-cell epitopes [34]. Alum is used conventionally as an adjuvant for diphtheria and tetanus toxoid vaccinations and transient IgE production has been demonstrated after treatment. There is some limited evidence that diphtheria and tetanus toxoid vaccination increases the risk of childhood clinical allergic disease. Calcium phosphate adjuvant may thus have some advantage in avoiding the potential pro-allergic bias of traditional alum.

Combination of physical with chemical modification is possible. Pyridine extract of non-defatted pollen has been precipitated with alum but the physical modification needed to achieve the allergoid may impair the ability of the allergen to induce IgG. Also grass pollen adsorption to L-tyrosine and modification with glutaraldehyde has produced fewer adverse events and a successful outcome after only three injections when compared with a conventional regime [35].

DNA oligodeoxynucleotides also function as an adjuvant if given with antigen. CpG (cytosine-phosphate-guanosine) motifs are commonly found in bacteria but are not common in vertebrates. Adaptive (lymphocytic) immunity as well as innate antibacterial activity are activated sequentially by CpG motifs in a Th1-directed manner [36]. APCs become activated and produce IL-6, TNF α and IL-12 whilst costimulatory surface molecules are upregulated on monocytes and dendritic cells. *In vivo* studies in mice have shown that B-cell mitosis is inhibited by CpG motifs [37]. Recently Kranzer et al. showed that in humans CpG motifs potentiate T cell activation by an APC-centred mechanism [37]. Furthermore, suppression of Th2 responses including IgE production may result from IFN γ or IL-10 production [38].

Conjugates of CpG DNA and allergen have been shown to produce a Th1 profile in mice that previously had measurable IgE against ragweed allergen [39]. DNA without CpG motifs is not effective while unconjugated allergen given with CpG DNA is not as effective, indicating that it is the conjugation of CpG DNA and allergen that is of importance at the APC interaction. Conventional immunotherapy regimes are limited by IgE-mediated side-effects but CpG allergen conjugate has less ability to bind IgE and release histamine than conventional allergen [40]. Direct comparison between CpG DNA therapy and conventional SIT is lacking and hindered by contrasting views on the mechanism of action of immunotherapy [41].

The Th1 IFN γ response to immunotherapy observed in mice reciprocally decreases Th2 IgE responses to allergen. This has been shown in some, but not all, human immunotherapy studies. In some human studies Th1 cytokines (IFN γ and IL-12) do not appear to be induced, whilst allergic symptoms decrease and Th2 cytokines are reduced [42]. Extensive studies on the mechanism of venom immunotherapy show evidence of regulatory T cell production and stimulation of macrophages to produce IL-10 which then inhibits Th2 proliferation [43]. This differential response between allergens implies CpG may only be of use in some allergies. The timing of CpG immunotherapy is important: starting treatment earlier on may prove to be more beneficial than treating a patient who has well-established disease. Potential problems with manifesting of a Th1 response have also been reported [44]. Eosinophilic airway inflammation and the formation of immune complexes of IFN- γ -dependent antibody isotypes are theoretical complications that remain to be investigated.

Oral administration of DNA vaccines is an exciting prospect. A murine model of oral immunotherapy has been successfully demonstrated by Roy et al. [45]. The main peanut allergen gene was cloned into an expression vector and administered orally to mice. Digestion was prevented by conjugation with chitosan, which is not digested proximally and adheres to intestinal walls where it is endocytosed. As outcome the authors measured faecal IgA, serum IgG_{2a} and specific IgE. The first two markers were elevated and the last decreased compared with placebo and control, indicating that the vaccine was immunologically active. Anaphylactic reactions induced in sensitized mice were also significantly milder in vaccinated compared with untreated mice. DNA vaccines thus appear safer and easier to administer than conventional SIT.

Peptides and IgE binding haptens

T cell-specific peptides are an attractive highly specific way of selecting T cell but not B cell responses. The conformational epitopes recognized by B cell receptors are not present if small linear peptides are synthesized and so IgE cross-linking and immediate hyper-sensitivity does not occur. Clinical trials in humans have been completed using Fel d 1 [46] and Amb a 1 [47]. Only a limited response was observed despite large injection doses (750 mcg) being administered. Outcome was measured by scoring of nasal and lung symptoms. A further beneficial effect of T cell peptides has been reported upon exposure to peptides without costimulatory input. This leads to decreased cytokine production and decreased cell surface molecule expression on Th1 and Th2 cells. Transient T cell activation may be the cause of skin and mucous membrane

side-effects observed in 16 of the 24 subjects investigated after receiving Fel d 1. MHC restriction of peptide recognition has implications for tailoring therapy to the individual and a recent cat peptide study has shown this to be clinically relevant. Accurate structural knowledge of the IgE binding sites of allergens has allowed the production of haptens which compete for IgE binding but do not cause linking of IgE and mast cell degranulation. Further *in vivo* trials are needed to establish the role of this mode of immunotherapy [48].

Future developments

DNA CpG motifs may be given in several different ways [49]. Alone, with allergen, with allergen DNA sequences or orally. Allergists have previously used heat-killed bacterial vaccines as immunotherapy adjuvants and these preparations would have undoubtedly included CpG motifs. Progress in this area could therefore not be defined as something completely new. 'Designer vaccines' using recombinant techniques will be more widely used in the future as mechanisms of SIT become more widely understood. When tailoring a DNA vaccine the tertiary structure of an allergen is defined by nuclear magnetic resonance or X-ray crystallography [50]. Epitopes may then be defined by protection assays or by mutations. The former method involves exposing a molecule to a monoclonal antibody followed by an isotope of water and the amino acid sequences protected by antibody are then identified. By either using predicted structures or trial and error modification of amino acids a molecule may be manufactured which does not bind to B cell epitopes [51]. Molecular breeding is a new technique that allows new genes and vectors to be produced and selected based upon function [52]. Allergens produced in this way have been demonstrated to show decreased skin test reactivity compared with native allergen.

More 'downstream' ways of halting the Th2 cytokine cascade downstream are a possible future therapeutic option. Anti-IL-4/IL-13 has recently been shown to reduce IgE production in mice [53] and anti-IL-5 has been reported to be of benefit in lowering eosinophil counts and decreasing bronchial hyper-responsiveness in guinea-pigs [54]. Human trials have been less promising however. A recent clinical trial of monoclonal antibody to IL-5, administered as a single injection at either high or low dose, decreased serum and sputum eosinophils significantly but did not affect the late asthmatic response or resulting allergen-induced bronchial hyper-responsiveness [55].

Non-specific proactive immunomodulation at an early age has been hypothesized by Holt as a way of primary prevention of any allergic disease [56]. The early postnatal period is most important in determining the risk of allergy

in future life and in the antigen-driven selection of a particular T cell population. If a Th1 milieu is predominant then Th2 responses are opposed. IL-2 and IFN γ -secreting Th1 cells result in non-pathogenic IgA and IgG responses, whilst Th2 cells secrete IL-4 and IL-5 and result in IgE and eosinophils as an immune response. Evidence for early life priming of the immune system exists and provides a mechanistic basis for Strachan's 'hygiene hypothesis' [57]; there is also much evidence that pregnancy is a Th2 state for mother and child and failure to resolve this in early life predisposes to childhood atopic disease. The route of exposure to allergens in early life is of prime importance as oral exposure to food allergens commonly produces a transient mild or sub-clinical IgE response. Later exposure and sensitization to airborne allergens does not produce such a transient clinical picture. Possible methods of producing a non-specific Th1 phenotype before primary sensitization occurs include: an allergen-bacterial protein preparation, a DNA plasmid vaccine, and oral vaccination strategies using transgenic foods containing genes to express potential allergens.

In children already established as atopic (with atopic dermatitis), oral *Lactobacillus rhamnosus* has been shown to elevate serum levels of IL-10 and this suggests a way of down-regulating IgE synthesis [58]. The value of such 'probiotics' in early life in children at high risk of allergic disease remains to be tested, although there is an increasing weight of observational evidence that a depleted microbial environment in the bowel in early life, either through diet or iatrogenically, is strongly associated with future atopic disease. Ethical considerations and the potential risk of enhancing delayed-type hypersensitivity and Th1 mediated disease must be of prime concern in this age group. A preventative interventional strategy must be able to accurately predict which individuals are at risk of atopic disease, but at the present time this is not yet possible. The onset of further sensitization has been prevented in atopic asthmatic children treated with *D. pteronyssinus* extract but further research is needed in this area to establish which individuals benefit most and whether any risks exist.

Non-specific immunomodulation in established adult allergy has recently been reported by means of recombinant IL-12 therapy. Serum and sputum markers of allergic disease (eosinophil counts) were reduced but objective measures of bronchial hyper-responsiveness and late asthmatic reaction were unaffected [59]. If an effective method of early life immunomodulation were to become available its use would be limited by our lack of ability to predict the natural history of childhood allergy and its relationship with physiological and serum markers of disease. Because of this, treating established allergy by immunotherapy remains the most viable therapeutic option for the near future. Progress may be in terms of fine-tuning

what is already available rather than something completely different.

References

- 1 Durham SR, Walker SM, Varga E-M et al. Long term clinical efficacy of grass pollen immunotherapy. *N Eng J Med* 1999; 341:468–75.
- 2 Graft DF, Golden DBK, Reisman RE, Valentine MD, Yunginger JW. The discontinuation of Hymenoptera venom immunotherapy. Report from the committee of insects of the American Academy of Allergy Asthma and Immunology. *J Allergy Clin Immunol* 1998; 101:573–5.
- 3 Barnes PJ. Is immunotherapy for asthma worthwhile? *N Eng J Med* 1996; 334:531–2.
- 4 Noon L. Prophylactic inoculation for hay fever. *Lancet* 1911; 1:1572.
- 5 Leynadier F, Banoun L, Dollois B et al. Immunotherapy with a calcium phosphate-adsorbed five-grass-pollen extract in seasonal rhinoconjunctivitis. *Clin Exp Allergy* 2001; 31:988–96.
- 6 Bosquet J, Lockey RF, Malling H-J. WHO position paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* 1999; 53:s6–s42.
- 7 Malling H, Weeke B. Immunotherapy. Position paper of the European Academy of Allergy and Clinical Immunology. *Allergy* 1993; 48 (Suppl. 14):9–35.
- 8 Varney VA, Gaga M, Frew AJ et al. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by anti-allergic drugs. *BMJ* 1991; 302:265–9.
- 9 Ewan PW, Alexander MM, Snape C et al. Effective hyposensitisation in allergic rhinitis using a potent partially purified extract of house dust mite. *Clin Allergy* 1988; 18:501–8.
- 10 Varney VA, Edwards J, Tabbah K et al. Clinical efficacy of specific immunotherapy to cat dander: a double blind placebo controlled study. *Clin Exp Allergy* 1997; 27:860–7.
- 11 Jacobsen L, Dreborg S, Moller C et al. Immunotherapy as preventative allergy treatment (PAT). *J Allergy Clin Immunol* 1996; 97:232.
- 12 Mosbech H, Osterballe O. Does the effect of immunotherapy last after the termination of treatment? Follow-up study in patients with grass pollen rhinitis. *Allergy* 1988; 43:523–9.
- 13 Golden DBK, Kagey-Sobotka A, Lichtenstein LM. Survey of patients after discontinuing venom immunotherapy. *J Allergy Clin Immunol* 2000; 105:385–90.
- 14 McHugh SM, Lavelle B, Kemeny DM et al. A placebo-controlled trial with two extracts of *D pteronyssinus* in allergic rhinitis, comparing clinical outcome with changes in IgE, IgG, and IgG subclasses. *J Allergy Clin Immunol* 1990; 86:521–32.
- 15 Robinson DS, Hamid Q, Ying S et al. Evidence for a predominant 'TH2-like' bronchoalveolar lavage T-lymphocyte population in atopic asthma. *N Eng J Med* 1992; 326:298–304.
- 16 Stewart GD, Lockey RF. Systemic reactions from allergen immunotherapy. *J Allergy Clin Immunol* 1992; 90:567–78.
- 17 Fell P, Brostoff J. A single dose desensitization for summer hay fever. *Eur J Clin Pharmacol* 1990; 38:77–9.
- 18 Birnbaum J, Charpin D, Vervloet D. Rapid Hymenoptera

- venom immunotherapy: comparative safety of three protocols. *Clin Exp Allergy* 1993; 23:226–39.
- 19 McHugh SM, Deighton J, Stewart AG et al. Bee venom immunotherapy induces a shift in cytokine responses from a TH2 to a TH1 dominant pattern: comparison of rush and conventional immunotherapy. *Clin Exp Allergy* 1995; 25:828–38.
 - 20 Crimi E, Voltolini S, Troise C et al. Local immunotherapy with Dermatophagoides extract in asthma. *J Allergy Clin Immunol* 1991; 87:721–8.
 - 21 Oppenheimer J, Areson JG, Nelson HS. Safety and efficacy of oral immunotherapy with standardized cat extract. *J Allergy Clin Immunol* 1994; 93:61–7.
 - 22 Sabbah A, Hassoun S, Le Sellin J et al. A double-blind, placebo-controlled trial by the sublingual route of immunotherapy with a standardized grass pollen extract. *Allergy* 1994; 49:309–13.
 - 23 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 J* 1998; 12:231–42.
 - 24 Akdis CA, Blesken T, Wymann D et al. Differential regulation of human T cell cytokine patterns and IgE and IgG4 responses by conformational antigen variants. *Eur J Immunol* 1998; 28:914–25.
 - 25 Vailes LD, Ying L, Bao Y et al. Fine specificity of B-cell epitopes on *Felis domesticus* allergen I (Fel d I): effect of reduction, alkylation and deglycosylation on Fel d I structure and antibody binding. *J Allergy Clin Immunol* 1994; 93:22–33.
 - 26 Olsson S, van Hage-Hamsten M, Whitley P. Contribution of disulphide bonds to antigenicity of Lep d 2, the major allergen of the dust mite *Lepidoglyphus destructor*. *Mol Immunol* 1998; 35:1017–23.
 - 27 Vrtala S, Hirtenlehner K, Vangelista L et al. Conversion of the major birch pollen allergen, Bet v 1, into two non-anaphylactic T cell epitope-containing fragments; candidates for a novel form of immunotherapy. *J Clin Invest* 1997; 99:1673–81.
 - 28 Smith AM, Chapman MD, Taketomi EA et al. Recombinant allergens for immunotherapy: a Der p 2 variant with reduced IgE reactivity retains T cell epitopes. *J Allergy Clin Immunol* 1998; 101:423–5.
 - 29 Passalacqua G, Albano M, Fregonese L et al. Randomised control trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet* 1998; 351:629–532.
 - 30 Marsh DG, Norman PS, Roebber M et al. Studies on allergoids from naturally occurring allergens. III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. *J Allergy Clin Immunol* 1981; 68:449–59.
 - 31 Bousquet J, Maasch HJ, Martinot B et al. Double-blind, placebo controlled immunotherapy with mixed grass allergoids. Comparison between parameters assessing the efficacy of immunotherapy. *J Allergy Clin Immunol* 1998; 82:439–46.
 - 32 McClure SJ, Wagland BM, Emery DL. Effect of Freund's adjuvant on local, draining and circulating lymphocyte populations in sheep. *Immunol Cell Biol* 1991; 69:361–7.
 - 33 Platts-Mills TAE, von Maur RK, Ishizaka K et al. IgA and IgG anti-ragweed antibodies in nasal secretions. *J Clin Invest* 1976; 57:1041–50.
 - 34 Grammer LC, Zeiss CR, Suszko IM et al. A double blind, placebo controlled trial of polymerized whole ragweed for immunotherapy of ragweed allergy. *J Allergy Clin Immunol* 1982; 69:494–9.
 - 35 Miller ACML, Tees EC. A metabolizable adjuvant: clinical trial of grass pollen-tyrosine adsorbate. *Allergy* 1974; 4:57–70.
 - 36 Chu RS, Targoni RS, Krieg AM et al. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (TH1) immunity. *J Exp Med* 1997; 186:1623–31.
 - 37 Kranzer K, Bauer M, Lipford GB et al. 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.
 - 38 Horner A, Takabayashi K, Burger J 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:S76.
 - 39 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.
 - 40 Kline JN, Businga TR, Jain V et al. Oral administration of CpG oligodeoxynucleotides provides protection against inflammation in asthma (Abstract). *J Resp Clin Care Med* 2000; 161:A21.
 - 41 Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998; 102:157–64.
 - 42 Kline JN, Krieg AM, Waldschmidt TJ et al. 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.
 - 43 Bellinghausen I, Metz G, Enk AH et al. 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.
 - 44 Sparwasser T, Miethke T, Lipford GB et al. Bacterial DNA causes septic shock. *Nature* 1997; 386:336–7.
 - 45 Roy K, Mao HQ, Huang SK et al. Oral gene delivery with chitanase-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nat Med* 1999; 5:387–91.
 - 46 Norman PS, Ohman JL, Long AA et al. Follow-up study of the first clinical trial with T cell defined peptides from cat allergen Fel d 1 (Abstract). *J Allergy Clin Immunol* 1995; 95:259.
 - 47 Creticos PS, Herbert J, Philips G et al. Efficacy of Allervax ragweed peptides in the treatment of ragweed-induced allergy (Abstract). *J Allergy Clin Immunol* 1997; 99:401.
 - 48 Ball T, Vrtala S, Sperr WR et al. Isolation of an

- immunodominant IgE hapten from an epitope expression cDNA library. Dissection of the allergic effector reaction. *J Biol Chem* 1994; 269:28323–8.
- 49 Heeg K, Zimmermann S. CpG DNA as a Th1 trigger. *Int Arch Allergy Immunol* 2000; 121:87–97.
- 50 Mueller GA, Smith AM, Hakkaart GAJ et al. Expression and secondary structure determination by NMR methods of the major house dust mite allergen Der p 2. *J Biol Chem* 1997; 272:26898–908.
- 51 Smith AM, Chapman MD. Localization of antigenic sites on Der p 2 using oligonucleotide-directed mutagenesis targeted to predicted surface residues. *Clin Exp Allergy* 1997; 27:593–9.
- 52 Punnonen J. Molecular breeding of allergy vaccines and antiallergic cytokines. *Int Arch Allergy Immunol* 2000; 121:173–82.
- 53 Carballido JM, Schols D, Namikawa R et al. IL-4 induces human B cell maturation and IgE synthesis in SCID-hu mice. Inhibition of ongoing IgE production by in vivo treatment with an IL-4/IL-13 receptor antagonist. *J Immunol* 1995; 155:4162–70.
- 54 Van Oosterhout AJ, Lidenjus R, Savelkhoul HF et al. Effect of anti-IL-5 and IL-5 on airway hyperreactivity and eosinophils in guinea pigs. *Am Rev Resp Dis* 1993; 147:548–52.
- 55 Leckie MJ, ten Brinke A, Khan J et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; 356:2144–8.
- 56 Holt PG. A potential vaccine strategy for asthma and allied atopic diseases during early childhood. *Lancet* 1994; 344: 456–8.
- 57 Strachan DP. Hay fever, hygiene and household size. *BMJ* 1989; 299:1259–60.
- 58 Pessi T, Sutas M, Hurme M et al. Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Clin Exp Allergy* 2000; 30:1804–8.
- 59 Bryan S, O'Connor B, Matti S et al. Effects of human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; 356:2149–53.

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