

13. Talmage, D. W. & Pearlman, D. S. The antibody response: a model based on antagonistic actions of antigen. *J. Theor. Biol.* **5**, 321–339 (1963).
14. Rajewsky, K. & Rottlander, E. Tolerance specificity and the immune response to lactic dehydrogenase isoenzymes. *Cold Spring Harb. Symp. Quant. Biol.* **32**, 547–554 (1967).
15. Mitchison, N. A. Antigen recognition responsible for the induction *in vitro* of the secondary response. *Cold Spring Harb. Symp. Quant. Biol.* **32**, 431–439 (1967).
16. Mitchison, N. A. Recognition of antigen by cells. *Prog. Biophys. Mol. Biol.* **16**, 3–14 (1966).
17. Russell, W. L. A high rate of somatic reversion in the mouse. *Genetics* **41**, 658 (1956).
18. Bretscher, P. A. & Cohn, M. Minimal model for the mechanism of antibody induction and paralysis by antigen. *Nature* **220**, 444–448 (1968).
19. Bretscher, P. The control of humoral and associative antibody synthesis. *Transplant. Rev.* **11**, 217–267 (1972).
20. Bretscher, P. & Cohn, M. A theory of self–nonself discrimination. *Science* **169**, 1042–1049 (1970).
21. Glick, B., Chang, T. S. & Jaap, R. G. The bursa of Fabricius and antibody production. *Poult. Sci.* **35**, 224–226 (1956).
22. Szenberg, A. & Warner, N. L. Dissociation of immunological responsiveness in fowls with a hormonally arrested development of lymphoid tissue. *Nature* **194**, 146–147 (1962).
23. Claman, H. N., Chaperon, E. A. & Triplett, R. F. Thymus–marrow–cell combinations — synergism in antibody production. *Proc. Soc. Exp. Biol. Med.* **122**, 1167–1171 (1966).
24. Mitchell, G. F. & Miller, J. F. A. P. Immunological activity of the thymus and thoracic-duct lymphocytes. *Proc. Natl Acad. Sci. USA* **59**, 296–303 (1968).
25. Mitchison, N. A. The carrier effect in the secondary response to hapten–protein conjugates. II. Cellular cooperation. *Eur. J. Immunol.* **1**, 18–27 (1971).
26. Boak, J. L., Mitchison, N. A. & Pattison, P. H. The carrier effect in the secondary response to hapten–protein conjugates. III. The anatomical distribution of helper cells and antibody-forming cell precursors. *Eur. J. Immunol.* **1**, 63–65 (1971).
27. Bretscher, P. A. A two-step, two-signal model for the primary activation of precursor helper T cells. *Proc. Natl Acad. Sci. USA* **96**, 185–190 (1999).
28. Lafferty, K. J. & Jones, M. A. S. Reactions of the graft-versus-host (GVH) type. *Aust. J. Exp. Biol. Med. Sci.* **47**, 17–54 (1969).
29. Simonsen, M. Graft-versus-host reactions. Their natural history and applicability as tools of research. *Prog. Allergy* **6**, 349–360 (1962).
30. Lafferty, K. J., Misko, I. S. & Cooley, M. A. Allogeneic stimulation modulates the *in vitro* response of T cells to transplantation antigen. *Nature* **249**, 275–276 (1974).
31. Lafferty, K. J., Cooley, M. A., Woolnough, J. A. & Walker, K. Z. Thyroid allograft immunogenicity is reduced after a period in organ culture. *Science* **18**, 259–261 (1975).
32. Talmage, D. W., Dart, G., Radovich, J. & Lafferty, K. J. Activation of transplant immunity: effect of donor leukocytes on thyroid allograft rejection. *Science* **191**, 385–388 (1976).
33. Lafferty, K. J. & Cunningham, A. J. A new analysis of allogeneic interactions. *Aust. J. Exp. Biol. Med. Sci.* **53**, 27–42 (1975).
34. Lafferty, K. J., Warren, H. S., Woolnough, J. A. & Talmage, D. W. Immunological induction of T lymphocytes: role of antigen and the lymphocyte costimulator. *Blood Cells* **4**, 395–404 (1978).
35. Baxter, A. G. *Germ Warfare: Breakthroughs in Immunology* (Allen and Unwin, Sydney, 2000).
36. Zinkernagel, R. M. & Doherty, P. C. Restriction of *in vitro* T-cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature* **248**, 701–702 (1974).
37. Cunningham, A. J. & Lafferty, K. J. A simple conservative explanation of the H-2 restriction of interactions between lymphocytes. *Scand. J. Immunol.* **6**, 1–6 (1977).
38. Lafferty, K. J., Andrus, L. & Prowse, S. J. Role of lymphokine and antigen in the control of specific T-cell responses. *Immunol. Rev.* **51**, 280–314 (1980).
39. Matzinger, P. Tolerance, danger and the extended family. *Annu. Rev. Immunol.* **12**, 991–1045 (1994).
40. Jenkins, M. K. & Schwartz, R. H. Antigen presentation by chemically modified splenocytes induces antigen-specific T-cell unresponsiveness *in vitro* and *in vivo*. *J. Exp. Med.* **165**, 302–319 (1987).
41. Nossal, G. J. & Pike, B. L. Clonal anergy: persistence in tolerant mice of antigen-binding B lymphocytes incapable of responding to antigen or mitogen. *Proc. Natl Acad. Sci. USA* **77**, 1602–1606 (1980).
42. Jenkins, M. K., Pardoll, D. M., Mizuguchi, J., Quill, H. & Schwartz, R. H. T-cell unresponsiveness *in vivo* and *in vitro*: fine specificity of induction and molecular characterisation of the unresponsive state. *Immunol. Rev.* **95**, 113–135 (1987).
43. Schwartz, R. H. In *Immunology: the Making of a Modern Science* (eds Gallagher, R. B., Gilder, J., Nossal, G. J. V. & Salvatore, G.) 95–101 (Academic, London, 1995).
44. Janeway, C. A. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb. Symp. Quant. Biol.* **54**, 1–13 (1989).
45. Janeway, C. A. The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunol. Today* **13**, 11–16 (1992).
46. Medzhitov, R., Preton-Hurlburt, P. & Janeway, C. A. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* **388**, 394–397 (1997).
47. Yang, R. B. *et al.* Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. *Nature* **395**, 284–288 (1998).
48. Yoshimura, A. *et al.* Cutting edge: recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. *J. Immunol.* **163**, 1–5 (1999).
49. Hayashi, F. *et al.* The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* **410**, 1099–1102 (2001).
50. Janeway, C. A. Presidential address to the American Association of Immunologists. *J. Immunol.* **161**, 539–544 (1998).
51. Rathmell, J. C., Townsend, S. E., Xu, J. C., Flavell, R. A. & Goodnow, C. C. Expansion or elimination of B cells *in vivo*: dual roles for CD40- and Fas (CD95)-ligands modulated by the B-cell antigen receptor. *Cell* **87**, 319–329 (1996).
52. Waldmann, H., Qin, S. & Cebbold, S. Monoclonal antibodies as agents to reinduce tolerance in immunity. *J. Autoimmun.* **5**, 93–102 (1992).
53. Gett, A. V. & Hodgkin, P. D. A cellular calculus for signal integration by T cells. *Nature Immunol.* **1**, 239–244 (2000).

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Online links

DATABASES

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OPINION

The future of antigen-specific immunotherapy of allergy

Rudolf Valenta

More than 25% of the population in industrialized countries suffers from immunoglobulin-E-mediated allergies.

The antigen-specific immunotherapy that is in use at present involves the administration of allergen extracts to patients with the aim to cure allergic symptoms. However, the risk of therapy-induced side effects limits its broad application. Recent work indicates that the epitope complexity of natural allergen extracts can be recreated using recombinant allergens, and hypoallergenic derivatives of these can be engineered to increase treatment safety. It is proposed that these modified molecules will improve the current practice of specific immunotherapy and form a basis for prophylactic vaccination.

Ninety years ago, long before the immunopathological mechanisms that underlie type I

(immunoglobulin-E-dependent) allergy were understood, Leonard Noon immunized patients suffering from pollen-induced hayfever with subcutaneous injections of pollen extracts¹. Although this was based on the erroneous belief that seasonal hayfever might be caused by a grass-pollen toxin, successful outcomes were recorded and induced protection was found to last for at least one year after the treatment was discontinued.

Since then, many of the underlying immunological and molecular mechanisms, as well as environmental factors, that influence the development of allergy have been analysed². Type I allergy is a classical IgE-mediated disease, which, as shown by a landmark experiment by Prausnitz and Küstner in 1921, requires at least three components: a disease-eliciting antigen (allergen); a transferable serum factor that discriminates allergic patients from healthy individuals (IgE);

and a tissue component that is present in all individuals (mast cells)³.

The antigens that elicit IgE responses are mostly proteins or glycoproteins that have molecular masses of 5–80 kDa, which elute easily from respirable particles and are highly immunogenic⁴. However, molecular and structural analyses of allergens from various sources indicate that there are no apparent common structural motifs or biological properties that predict their allergenic nature. It is now becoming clear that the increased susceptibility

of allergic individuals to mount IgE responses compared with non-allergic individuals — termed atopy — is also controlled by several genetic factors^{5–7}.

Sensitization

In allergic individuals, specific IgE antibodies can be detected in the first few years after birth, and seem to be induced by early, post-natal allergen contact at mucosal surfaces^{8–10}. This primary response to allergen is known as sensitization (FIG. 1a). In the case of respiratory

allergies, minute amounts of soluble allergens (in the ng range) are released from allergen-bearing particles (for example, pollen) on mucosal surfaces. In this context, it has been shown that the microenvironment of the respiratory tract promotes the differentiation of CD4⁺ T cells to T helper 2 (T_H2) cells during T-cell priming¹¹. Although it has not been possible yet to study the events of primary sensitization in children who become allergic, studies in mouse models indicate that antigen-presenting cells (for example, dendritic cells;

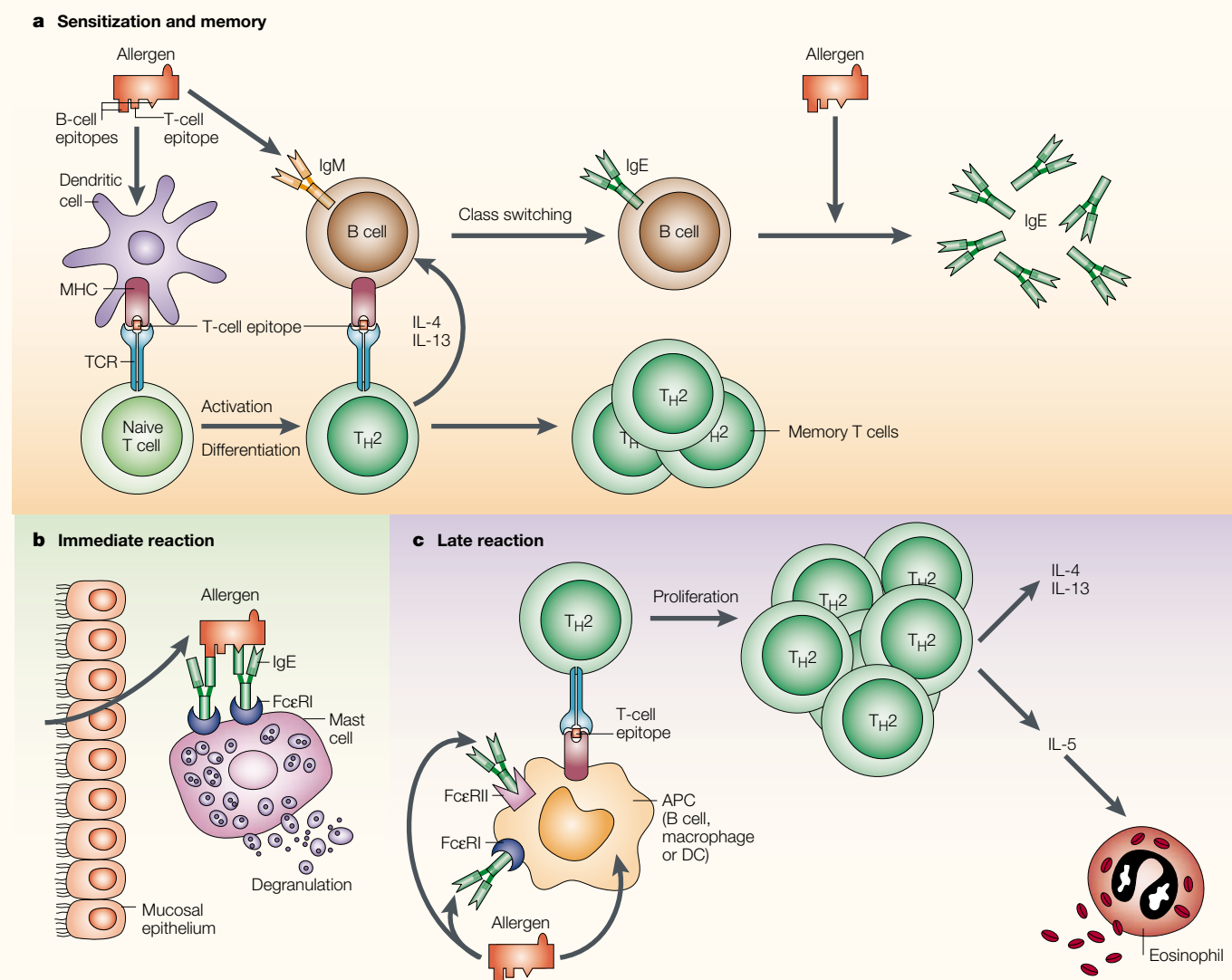


Figure 1 | How allergens induce and maintain allergy. **a** | Sensitization and memory. Initial contact with minute amounts of intact, soluble allergen at mucosal surfaces, particularly of the respiratory tract, might favour allergen uptake by potent antigen-presenting cells (for example, dendritic cells) and/or immunoglobulin-mediated capture by specific B cells. If T helper 2 (T_H2)-cell help is acquired, cytokines such as interleukin-4 (IL-4) and IL-13 will be produced that favour immunoglobulin-class switching of specific B cells to immunoglobulin E (that is, sensitization). Sensitization leads to the establishment of IgE⁺ memory B cells and allergen-specific memory T cells. Subsequent repeated allergen contact will boost IgE⁺ memory B cells that receive T-cell help to produce increased levels of allergen-specific IgE antibodies. These are loaded by means of specific receptors (FcεRI, high-affinity IgE receptor; FcεRII, low-affinity IgE receptor) onto mast cells, basophils, monocytes, dendritic cells and B cells. **b** | Immediate reaction. The crosslinking of effector-cell-bound IgE by allergens leads to the release of biologically active mediators (histamine, leukotrienes) by means of degranulation and, so, to the immediate symptoms of allergy. **c** | Late reaction. This is caused by the presentation of allergens to T cells, which become activated, proliferate and release proinflammatory cytokines (for example, IL-4, IL-5 and IL-13). This process might be enhanced by the IgE-mediated presentation of allergens to T cells. T_H2 cytokines (for example, IL-5) induce tissue eosinophilia and the release of inflammatory mediators from eosinophils. APC, antigen-presenting cell; DC, dendritic cell; TCR, T-cell receptor.

DCs) take up allergens and prime allergen-specific T cells, which can then help to activate allergen-specific B cells by means of cognate T-cell–B-cell interactions. This possibility is supported by a body of literature that shows that DCs can stimulate potent T_H2 responses, and by a report that shows that lung DCs, but not B cells, stimulate strong ovalbumin-specific T-cell responses in mice^{12,13}.

T_H2 cells produce cytokines — such as interleukin-4 (IL-4) and IL-13 — that promote the immunoglobulin-class switching of B cells to IgE (FIG. 1a). The administration of IL-4 antagonists during primary sensitization prevents the development of IgE responses in mice, which shows the importance of IL-4 in controlling the class switch towards IgE production¹⁴. Continuing clinical trials are investigating the use of IL-4 and IL-13 antagonists for the treatment of allergic patients¹⁵.

In addition to IL-4 and IL-13, various other factors influence the differentiation of $CD4^+$ T cells to the T_H2 phenotype, including the genetic background of the individual, the site of allergen contact, allergen dose and conformation, the nature and number of antigen-presenting cells, and the extent of T-cell receptor (TCR) ligation, as well as biological mediators (for example, histamine)^{16–20}. As a result of sensitization, allergic patients produce IgE antibodies that are specific for allergens (FIG. 1a); by contrast, low levels of allergen-specific IgG antibodies are found in both allergic and non-allergic individuals¹⁰.

After sensitization, allergen-specific T cells form a pool of long-lived memory T cells that respond to repeated allergen contact²¹; these cells can be detected in the skin and peripheral blood of sensitized individuals by sequence

analysis of their TCR hypervariable regions^{22,23} (FIG. 1a). The sensitization process (that is, the primary response) also leads to the establishment of an allergen-specific IgE antibody memory¹⁰. Interestingly, allergen-specific IgE⁺ antibody-producing cells are found in the mucosa of the respiratory tract (nasal and bronchial mucosa), where they are in contact with inhaled allergens^{24–26}, but they are also present in the peripheral blood of allergic patients^{27–29}. Levels of allergen-specific IgE and the sensitivity of patients to allergens are strongly enhanced 2–3 weeks after seasonal allergen contact³⁰ or nasal provocation with allergens³¹ (V. Niederberger and R.V., unpublished observations). This finding, together with the fact that allergen contact is a potent stimulus for allergen-specific IgE production, indicates that allergen-specific IgE in allergic patients is produced mainly by IgE⁺ memory B cells, which are strongly activated by contact with the allergen itself to produce increased levels of antibody (FIG. 1a). The available evidence indicates that these allergen-specific IgE⁺ memory B cells must receive help from allergen-specific T cells to be activated. As has been shown for other disease models, it is also possible that certain subsets of regulatory T cells might be able to regulate allergen-specific antibody production and T-cell responses^{32–34}.

The immediate-phase reaction

It is becoming increasingly evident that IgE antibodies can have diverse pathological effects. It is well established that IgE binds tightly, by means of the high-affinity receptor FcεRI, to mast cells and basophils³⁵. Cross-linking of receptors by allergen-bound IgE

induces the release of inflammatory mediators (for example, histamine and leukotrienes) which, in turn, cause, within minutes, the immediate symptoms of disease, such as rhinitis, conjunctivitis and asthma (FIG. 1b). Studies to analyse the mechanisms of action of anti-IgE-based therapies have now confirmed that for basophils, and perhaps also for mast cells, IgE antibodies are potent upregulators of the expression of FcεRI and the lower-affinity FcεRII (CD23) on the cell surface^{36–39}. Moreover, there is evidence that the binding of monomeric IgE might increase the survival of mast cells by preventing their apoptosis^{40,41}. Similar observations have been made for monocytes, which seem to escape apoptosis after IgE-mediated FcεRI ligation⁴². The expression of FcεRI has also been shown on various other cell types (such as eosinophils, thrombocytes and epithelial cells), but whether this contributes to the pathology of allergy is either a controversial issue or has not been investigated yet^{43–48}. Recently, evidence was provided that regulatory T cells might influence mast-cell activity directly⁴⁹. Conversely, it has been found that histamine, a mediator that is released by mast cells and basophils, can regulate T-cell responses by means of interactions with specific receptors⁵⁰.

The late-phase reaction

In allergic individuals that suffer from chronic manifestations of atopy (for example, chronic asthma and atopic dermatitis), late-phase reactions — which are caused by the activation of allergen-specific T cells after hours to days — are pronounced (FIG. 1c). These are characterized by a strong T-cell infiltration and eosinophil activation. A role for IgE antibodies in late-phase reactions has been suggested on the basis of several *in vitro* studies that show that antigen-presenting cells (for example, B cells, monocytes and DCs) can bind IgE antibodies by means of specific receptors (FcεRI and FcεRII), and that the presentation of allergens that are taken up by means of receptor-bound IgE antibodies is more potent at activating allergen-specific T-cell responses than antigen presentation that results from nonspecific antigen uptake^{51–54}. This indicates that allergen-specific IgE antibodies might have an unexpected role in the activation of allergen-specific T cells, by means of facilitating antigen presentation (FIG. 1c). However, a recent study has shown that the activation of allergen-specific T cells can be achieved directly by T-cell-epitope-containing peptides that lack epitopes for binding to IgE⁵⁵. This observation clearly shows that allergens can activate T cells in an IgE-independent manner and that these

Table 1 | Potential mechanisms of antigen-specific therapy of allergy

Targeted process	Mechanism	References
Immediate-phase reactions		
Immediate skin reactions (mast-cell degranulation)	Blocking IgG competing with IgE	68
Basophil degranulation	Blocking IgG competing with IgE	64,71
Mast-cell number	Reduction by unknown mechanism	120
Mediator release from mast cells and basophils	Reduction by T-cell-derived IL-10 and IFN-γ	119
Late-phase reactions		
IgE-mediated allergen presentation to T cells, T-cell activation and cytokine release	Blocking IgG competing with IgE	72
T-cell proliferation	Generation of suppressor cells; T-cell tolerance/anergy	73 77–81,84,85,118
T_H2 cytokine production	Reduction of T-cell-derived IL-4 and IL-13	74–77,81,116
T_H1 cytokine production	Induction of T_H1 cells	75–77
Eosinophil number	IL-5 reduction	117

IFN-γ, interferon-γ; Ig, immunoglobulin; IL, interleukin; T_H , T helper.

allergen-specific T cells are important for the induction of late-phase symptoms (FIG. 1c). A possible role for DCs in the regulation of late-phase reactions is indicated by the finding that, depending on the cytokine milieu, DCs can either induce tolerance to respiratory antigens or strongly activate T_H2 responses and eosinophils^{34,56,57}. Eosinophil activity depends mainly on **IL-5**, and it is known that these cells can produce various inflammatory mediators and immunoregulatory cytokines that are implicated in the late asthmatic response^{58–60}. Two recent clinical studies using recombinant human **IL-12**, which is known to suppress eosinophilic inflammation, and neutralizing IL-5 antibodies for the treatment of patients with allergic asthma have shown a strong reduction of tissue eosinophilia, but no effects on airway hyperresponsiveness and late asthmatic responses^{61,62}.

Immunotherapy of allergy

The differentiation, phenotype and activity of allergen-specific T cells are controlled by various allergen-specific factors, including antigen dose, antigen conformation and the site of antigen exposure^{11,16,63}. It is, therefore, possible to modulate allergen-specific T cells using specific antigen. This, together with the finding that the profile of allergens and epitopes that are recognized by IgE does not change substantially during the natural course of allergic disease⁶⁴, is an excellent basis for the antigen-targeted immunotherapy of allergy.

The clinically most effective and frequently used form of immunotherapy of allergy is injection immunotherapy⁶⁵. It involves repeated subcutaneous injections of increasing doses of adjuvant-bound allergen extracts. Numerous clinical studies have documented the efficacy of immunotherapy, but the underlying immunological mechanisms remain a matter of controversy^{65,66}. The potential mechanisms that have been identified are summarized in TABLE 1. In 1935, more than 30 years before the discovery of IgE antibodies, Robert A. Cooke provided the first insight into the mechanisms of injection immunotherapy by showing that the transfusion of blood from a patient who had been successfully treated with immunotherapy to an allergic recipient cured hayfever in the latter⁶⁷. A few years later, the transferable factor was identified as IgG antibodies, which, owing to their ability to inhibit immediate skin reactions to allergen provocation, were designated blocking antibodies⁶⁸. However, the importance of blocking antibodies has been questioned on the basis of the finding that the induction of IgG antibodies is not always associated with clinically successful immunotherapy⁶⁹. Nevertheless, some

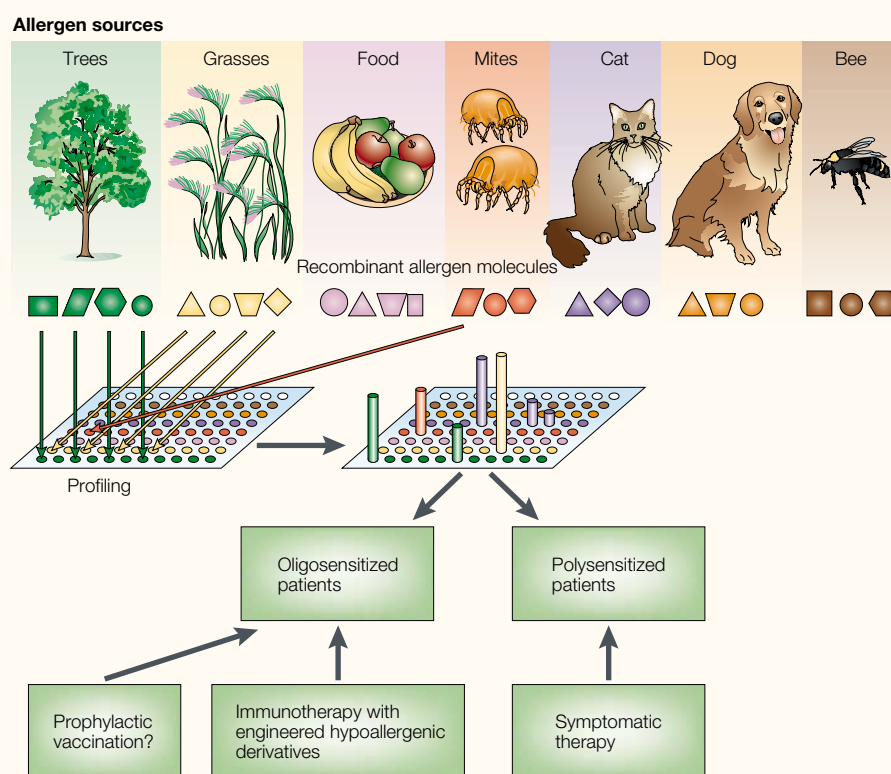


Figure 2 | Assembling complete panels of hypoallergenic allergen derivatives for prophylactic vaccination against allergy. Recombinant allergens that contain most of the relevant epitopes that are present in various allergen sources have been produced by molecular cloning. The recombinant allergen molecules can be used to establish multi-allergen test systems (for example, allergen microarrays) that facilitate the determination of the reactivity profile of an allergic patient. On the basis of this information, it is possible to discriminate between those allergic patients who are sensitized against a few allergen components (oligosensitized patients) and those who react against many allergen components (polysensitized patients). Antigen-targeted immunotherapy of established allergy in oligosensitized patients can be carried out according to their reactivity profiles, whereas polysensitized patients might benefit more from symptomatic treatment. At present, hypoallergenic allergen derivatives are being developed for most of the important allergens, which can then be used for patient-tailored immunotherapy of oligosensitized patients with a reduced risk of inducing anaphylactic side effects. If hypoallergenic derivatives can be developed that comprise most of the relevant allergen sources, it will be possible to assemble prophylactic allergy vaccines.

recent studies carried out in defined molecular and cellular systems have rekindled interest in the concept of blocking antibodies^{64,70–72}. First, it has been shown that immunotherapy-induced IgG antibodies (mainly IgG₁ and IgG₄)⁷¹ compete with IgE for their binding sites on the allergens; this prevents the allergen-induced activation of basophils and, so, the release of biological mediators^{64,71}. Second, it has been shown that blocking antibodies strongly inhibit the IgE-mediated presentation of allergens to T cells, which might reduce T_H2 activation and the subsequent release of T_H2 cytokines⁷².

Several findings indicate an important role for T cells in successful immunotherapy. They include reports of the generation of suppressor T cells during immunotherapy⁷³, the observation that immunotherapy decreases the production of IL-4 by CD4⁺ T cells^{74,75}

and the finding that immunotherapy shifts cytokine responses from a T_H2 to a T_H1 pattern^{76,77}. The induction of T-cell tolerance has also been suggested to be a possible mechanism of immunotherapy^{78,79}. In this context, attempts have been made to use T-cell-epitope-bearing peptides that lack IgE-binding sites for immunotherapy. In mouse models, it was found that T-cell-epitope-containing peptides induced tolerance^{80,81}, but the evaluation of T-cell-epitope-containing peptides in clinical studies has produced controversial results. Although it was found in one study that immunotherapy with peptides derived from the major cat allergen **Fel d 1** reduced the release of IL-4 by cultured peripheral-blood mononuclear cells from treated patients⁸², no effects on cytokine production, or immediate- or late-phase skin reactivity were observed in another study⁸³. A recent

study that reinvestigated T-cell-epitope-containing peptides has shown a suppression of T-cell-mediated late-phase responses that was preceded by T-cell activation⁸⁴, and another study reported the induction of T-cell anergy in allergic patients that were treated with T-cell-epitope-containing peptides of **bee-venom phospholipase**⁸⁵.

It has not been determined yet whether immunotherapy-induced blocking antibodies or T-cell-based downregulation can prevent the continuous boosting of IgE production, which otherwise might occur in allergic patients through repeated allergen contact. It is probable that several different mechanisms are responsible for the observed long-term effects (that is, prolonged clinical remission of symptoms) of injection immunotherapy and for the clinical observation that this form of allergen-specific immunotherapy can prevent the progression of allergic disease from less-severe (such as hayfever) to more-severe symptoms (such as asthma)^{65,66,86}.

Disadvantages of immunotherapy

One of the main problems of immunotherapy is that the administration of allergens might cause local and systemic side effects in patients⁴, such as urticaria, asthma attacks and life-threatening anaphylactic shock. Therefore, several attempts have been made to reduce therapy-induced anaphylactic side effects. They include the adsorption of allergen extracts to adjuvants to delay the systemic release of allergenic material from the site of administration and to increase the immunogenicity of the vaccine^{65,87}, and the chemical modification of allergen extracts to reduce their allergenic activity^{65,88,89}.

Another problem concerning current forms of immunotherapy relates to the use of allergen extracts for diagnosis and therapy. Allergen extracts are obtained from natural allergen sources. They contain various allergenic, as well as non-allergenic, components and might even be contaminated with allergens from other sources⁹⁰. Furthermore, the allergenic components of an allergen extract might have different levels of immunogenicity, and the amount of allergen in the diagnostic and therapeutic extract preparation can vary markedly⁶⁵. Diagnoses using allergen extracts can identify allergen sources, but they cannot identify the disease-eliciting allergen molecules⁹¹. So, it is impossible to determine using allergen extracts whether a patient is co-sensitized towards several allergen sources or is cross-sensitized to an allergen that is present in different allergen sources (for example, profilin)⁹². Another potentially important problem related to the

unpredictable complexity of allergen extracts is that therapy-induced new IgE reactivities towards extract components that were not recognized before therapy can arise after treatment^{71,93}.

Cloning complete allergen repertoires

During the past decade, several research groups have succeeded in isolating and cloning complementary DNAs that encode most of the clinically relevant allergen molecules. These clones have been used to produce panels of recombinant allergens that reproduce the epitope complexity that is present in natural allergen extracts⁹⁴ (FIG. 2). Whether recombinant allergens contain most of the relevant IgE epitopes that are present in natural allergen sources can be easily tested by immunological competition experiments. For example, one can test whether a given panel of recombinant allergens is sufficient to deplete most of the IgE antibodies that are specific for natural allergens from the sera of allergic patients^{95–97}.

“...if prophylactic treatment can be given early in life, it might be possible to prevent the development of allergy.”

The availability of recombinant allergens also opens new opportunities for refined allergy diagnosis. Whereas previously, only allergen-containing sources could be defined, it is now possible to identify the disease-eliciting allergen molecules and, so, determine and monitor the reactivity profile of a patient by component-resolved diagnosis (CRD)⁹¹ with recombinant allergen molecules (FIG. 2). Using allergen molecules that are present in the most common allergen sources, miniaturized allergy tests have been developed that are based on microarray technology⁹⁴. It is conceivable that such test systems will allow, in the near future, the determination of reactivity profiles for allergic patients to large numbers of disease-causing allergens and epitopes, by means of a single assay that requires only minute amounts of serum.

According to the test results that are obtained by CRD, it will be possible to select patients more effectively for specific immunotherapy. For example, it will enable oligosensitized patients (who would benefit from immunotherapy treatment) to be distinguished from those patients who react against

many allergen components and, hence, might be better treated by pharmacological therapy. Extract-based immunotherapy can be monitored by recombinant-allergen-based assays to detect new sensitizations, which might allow treatment to be stopped before the new sensitizations become clinically relevant^{71,93}.

‘Detoxifying’ allergens

The application of recombinant-DNA technology to allergen characterization has revealed the molecular nature of many important allergens and has advanced the characterization of their immunological and structural features⁴. On the basis of this work, it has become possible to modify important allergens in ways that simultaneously reduce allergenic activity and preserve relevant T-cell epitopes and structures that are necessary for the induction of antibody responses⁹⁸. Such hypoallergenic allergen derivatives have been generated mainly by recombinant-DNA technology by modifying the allergen-encoding cDNA or by synthetic peptide chemistry^{99–109}. Hypoallergenic allergen derivatives have two main advantages. First, it is possible to reduce their allergenic activity in a defined and reproducible manner. Second, they can be engineered to target T cells and B cells simultaneously. The IgE-binding capacity and ability of these molecules to induce IgE-mediated immediate reactions has often been reduced by the destruction of conformational IgE epitopes — by the production of recombinant allergen fragments^{103,107,108} or by introducing mutations into the molecules by site-directed mutagenesis^{100,102,104,105}. A strong reduction in allergenic activity and the simultaneous preservation of T-cell and B-cell epitopes has also been obtained by recombinant oligomerization¹⁰⁶. Unexpectedly, it was observed that some of the recombinant hypoallergenic derivatives had markedly altered immunogenic properties that might be advantageous for immunotherapy (for example, increased immunogenicity and increased induction of T_H1 cytokines)^{106,108,109}. Molecular ‘architects’ can now engineer and evaluate a wide variety of hypoallergenic allergen derivatives. They can compare the modified allergens with wild-type allergens in terms of their IgE-binding capacity, their ability to induce lymphoproliferative and cytokine responses, and their potential to induce the release of biological mediators from effector cells^{106,107}. Derivatives that have reduced allergenic activity *in vitro* are evaluated further for their ability to prevent or treat allergy in experimental animal models^{106–108,110}. Recombinant hypoallergenic derivatives of the main birch-pollen allergen **Bet v 1** have already been evaluated in

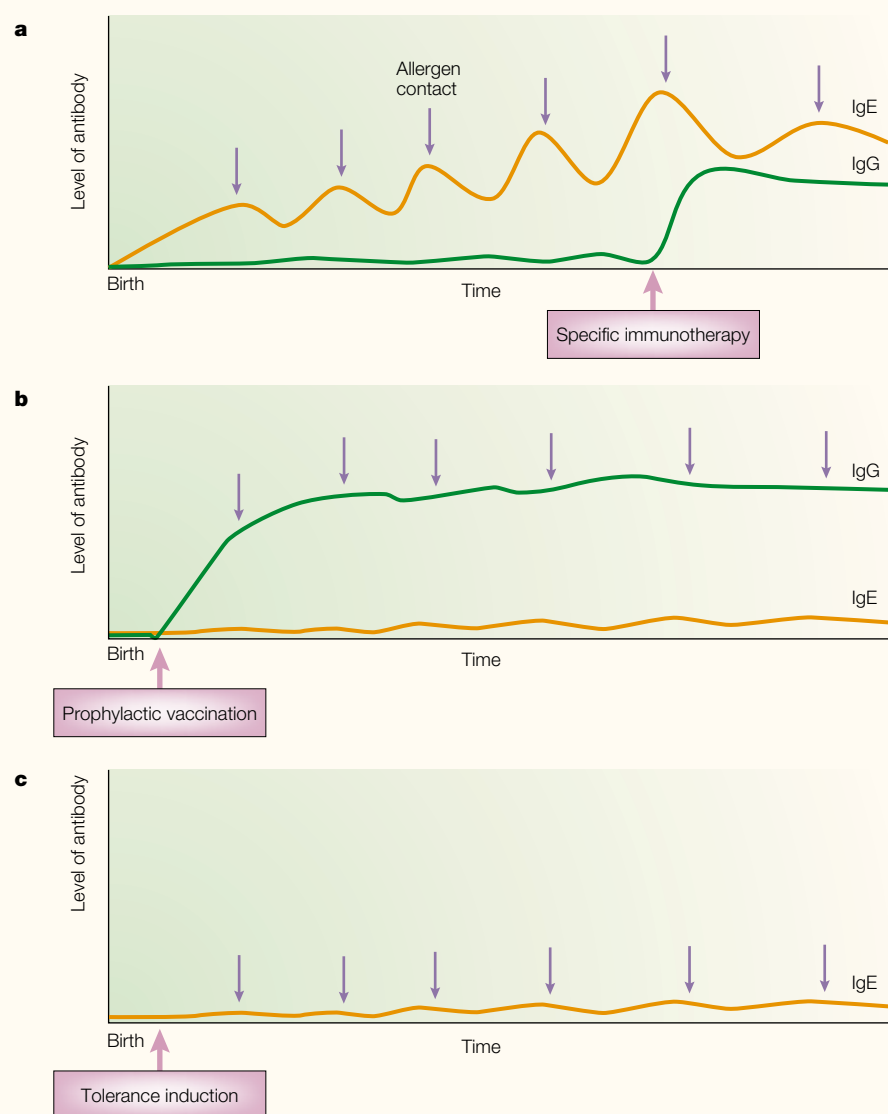


Figure 3 | Proposed action of prophylactic allergy vaccines. a | In allergic individuals, sensitization occurs shortly after birth due to allergen contact. Repeated allergen contact during life might continuously boost allergen-specific immunoglobulin E production and, hence, might be responsible for the aggravation and spreading of allergic symptoms. In sensitized patients, allergen-specific IgG levels are low. Immunotherapy that counteracts established allergy is mostly associated with the strong induction of allergen-specific IgG antibodies and T-cell modulation. **b** | Prophylactic vaccination with hypoallergenic allergen derivatives shortly after birth might induce high levels of allergen-specific IgG antibodies, which will form complexes with allergens and, hence, probably prevent sensitization and/or the boosting of allergen-specific IgE production by natural allergen exposure. Consequently, IgE responses will not develop or will remain at low levels. **c** | Tolerance induction with hypoallergenic allergen derivatives early in life might prevent the development of IgE responses that otherwise would occur after allergen contact.

terms of their *in vivo* allergenic activity by skin testing of allergic patients^{111–114}, and some of the derivatives have been evaluated recently in a double-blind, placebo-controlled, multicentre (Vienna, Strasbourg and Stockholm) immunotherapy study of patients who are allergic to birch pollen.

Towards prophylactic vaccination

The conventional immunotherapy of allergic patients faces the inherent difficulty of

attempting to counteract an already established pathological immune response. In addition to the treatment of established allergy, it might be necessary to consider prophylactic vaccination against allergy before initial sensitization has taken place and strong IgE responses and T-cell priming have been established. In untreated allergic patients, IgE and T-cell responses can be boosted continuously by allergen contact and, in severe cases, might lead to the inevitable progression of

the disease from moderate to severe symptoms of atopy (for example, asthma) (FIG. 3a). However, if prophylactic treatment can be given early in life, it might be possible to prevent the development of allergy¹¹⁵. This might be achieved by prophylactic vaccination to induce 'normal', non-IgE-mediated, allergen-specific immune responses that are associated with the production of IgG antibody using protocols similar to those that are used for vaccination against infectious diseases (FIG. 3b). Alternatively, it might be possible to use recombinant hypoallergenic allergen derivatives for the induction of T-cell tolerance to prevent the development of allergen-specific immune responses^{110,115} (FIG. 3c).

Both prophylactic vaccination and tolerance induction against most allergen sources will require that a reasonably complete repertoire of relevant allergens can be assembled — a goal that, in view of the rapid progress in the field of molecular allergen characterization and recombinant allergens (see Further Information websites), seems feasible. To avoid unwanted sensitizations that might occur eventually during the course of prophylactic treatment, it will be necessary to use hypoallergenic derivatives that do not share IgE-binding epitopes with the wild-type allergens. Owing to the low allergenic activity of the modified allergens and the lack of a pre-existing IgE response to the derivatives in the individuals to be treated, it will be possible to administer high doses similar to those that are used in vaccination and tolerance-induction protocols. The fact that the current practice of vaccinating against infectious agents in early childhood does not induce allergic sensitization should mitigate any concern that prophylactic vaccination with hypoallergenic allergen derivatives might induce allergy. Nevertheless, experimental animal studies and clinical pilot studies will be required to assess the potential risks of prophylactic allergy vaccination. First, clinical studies might be conducted in non-atopic adults to exclude unwanted side effects (for example, the induction of allergic sensitization). If encouraging results are obtained and safety has been proven, it might be possible to vaccinate children that have a genetic predisposition for developing allergy.

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1. Noon, L. Prophylactic inoculation against hay fever. *Lancet* **1**, 1572–1573 (1911).
2. Wills-Karp, M., Santeliz, J. & Karp, C. L. The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nature Rev. Immunol.* **1**, 69–75 (2001).
3. Prausnitz, C. & Küstner, H. Studien über die Überempfindlichkeit. *Centralbl. f. Bakteriol.* **86**, 160–169 (1921).
4. Valenta, R. & Kraft, D. Recombinant allergen molecules: tools to study effector-cell activation. *Immunol. Rev.* **179**, 119–127 (2001).
5. Daniels, S. E. *et al.* A genome-wide search for quantitative trait loci underlying asthma. *Nature* **383**, 247–250 (1996).
6. Lonjou, C. *et al.* A first trial of retrospective collaboration for positional cloning in complex inheritance: assay of the cytokine region on chromosome 5 by the consortium on asthma genetics (COAG). *Proc. Natl Acad. Sci. USA* **97**, 10942–10947 (2000).
7. Lee, Y. A. *et al.* A major susceptibility locus for atopic dermatitis maps to chromosome 3q21. *Nature Genet.* **26**, 470–473 (2000).
8. Wahn, U. *et al.* Indoor allergen exposure is a risk factor for sensitization during the first three years of life. *J. Allergy Clin. Immunol.* **99**, 763–769 (1997).
9. Kulig, M. *et al.* Natural course of sensitization to food and inhalant allergens during the first six years of life. *J. Allergy Clin. Immunol.* **103**, 1173–1179 (1999).
10. Niederberger, V., Niggemann, B., Kraft, D., Spitzauer, S. & Valenta, R. Evolution of IgM, IgE and IgG₁₋₄ antibody responses in early childhood monitored with recombinant allergen components: implications for class-switch mechanisms. *Eur. J. Immunol.* **32**, 576–584 (2002).
11. Constant, S. L., Lee, K. S. & Bottomly, K. Site of antigen delivery can influence T-cell priming: pulmonary environment promotes preferential T_H2-type differentiation. *Eur. J. Immunol.* **30**, 840–847 (2000).
12. Lambrecht, B. N. The dendritic cell in allergic airway diseases: a new player to the game. *Clin. Exp. Allergy* **31**, 206–218 (2001).
13. Masten, B. J. & Lipscomb, M. F. Comparison of lung dendritic cells and B cells in stimulating naive antigen-specific T cells. *J. Immunol.* **162**, 1310–1317 (1999).
14. Grunewald, S. M. *et al.* An antagonistic IL-4 mutant prevents type I allergy in the mouse: inhibition of the IL-4/IL-13 receptor system completely abrogates humoral immune responses to allergen and development of allergic symptoms *in vivo*. *J. Immunol.* **160**, 4004–4009 (1998).
15. Borish, L. C. *et al.* Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J. Allergy Clin. Immunol.* **107**, 963–970 (2001).
16. Akdis, C. A., Blesken, T., Wymann, D., Akdis, M. & Blaser, K. Differential regulation of human T-cell cytokine patterns and IgE and IgG₄ responses by conformational antigen variants. *Eur. J. Immunol.* **28**, 914–925 (1998).
17. Tanaka, H., Demeure, C. E., Rubio, M., Delespesse, G. & Sarfati, M. Human monocyte-derived dendritic cells induce naive T-cell differentiation into T helper cell type 2 (Th2) or Th1/Th2 effectors. Role of stimulator/responder ratio. *J. Exp. Med.* **192**, 405–412 (2000).
18. Constant, S., Pfeiffer, C., Woodard, A., Pasqualini, T. & Bottomly, K. Extent of T-cell receptor ligation can determine the functional differentiation of naive CD4⁺ T cells. *J. Exp. Med.* **182**, 1591–1596 (1995).
19. Jahnsen, F. L. *et al.* Experimentally induced recruitment of plasmacytoid (CD123^{high}) dendritic cells in human nasal allergy. *J. Immunol.* **165**, 4062–4068 (2000).
20. Mazzoni, A., Young, H. A., Spitzer, J. H., Visintin, A. & Segal, D. M. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T-cell polarization. *J. Clin. Invest.* **108**, 1865–1873 (2001).
21. Chakir, J., Laviolette, M., Turcotte, H., Boutet, M. & Boulet, L. P. Cytokine expression in the lower airways of nonasthmatic subjects with allergic rhinitis: influence of natural allergen exposure. *J. Allergy Clin. Immunol.* **106**, 904–910 (2000).
22. Van Reijssen, F. C., Bruijnzeel-Koomen, C. A., de Weger, R. A. & Mudde, G. C. Retention of long-lived, allergen-specific T cells in atopic dermatitis skin. *J. Invest. Dermatol.* **108**, 530 (1997).
23. Bohle, B. *et al.* Long-lived Th2 clones specific for seasonal and perennial allergens can be detected in blood and skin by their TCR hypervariable regions. *J. Immunol.* **160**, 2022–2027 (1998).
24. Durham, S. R. *et al.* Expression of ϵ germ-line gene transcripts and mRNA for the ϵ heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. *Eur. J. Immunol.* **27**, 2899–2906 (1997).
25. Ying, S. *et al.* Local expression of ϵ germ-line gene transcripts and RNA for the ϵ heavy chain of IgE in the bronchial mucosa in atopic and nonatopic asthma. *J. Allergy Clin. Immunol.* **107**, 686–692 (2001).
26. Smurthwaite, L. *et al.* Persistent IgE synthesis in the nasal mucosa of hay-fever patients. *Eur. J. Immunol.* **31**, 3422–3431 (2001).
27. Steinberger, P. *et al.* Allergen-specific IgE production of committed B cells from allergic patients *in vitro*. *J. Allergy Clin. Immunol.* **96**, 209–218 (1995).
28. Dolecek, C. *et al.* Effects of IL-4 and IL-13 on total and allergen-specific IgE production by cultured PBMC from allergic patients determined with recombinant pollen allergens. *Clin. Exp. Allergy* **25**, 879–889 (1995).
29. Steinberger, P., Kraft, D. & Valenta, R. Construction of a combinatorial IgE library from an allergic patient: isolation and characterization of human IgE Fabs with specificity for the major timothy grass-pollen allergen, Phl p 5. *J. Biol. Chem.* **271**, 10967–10972 (1996).
30. Henderson, L. L., Larson, J. B. & Gleich, G. J. Maximal rise in IgE antibody following ragweed pollination season. *J. Allergy Clin. Immunol.* **55**, 10–15 (1975).
31. Naclerio, R. M. *et al.* Nasal provocation with allergen induces a secondary serum IgE antibody response. *J. Allergy Clin. Immunol.* **100**, 505–510 (1997).
32. Groux, H. *et al.* A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **389**, 737–742 (1997).
33. Thornton, A. E. & Shevach, E. M. CD4⁺CD25⁺ immunoregulatory T cells suppress polyclonal T-cell activation *in vitro* by inhibiting interleukin-2 production. *J. Exp. Med.* **188**, 287–296 (1998).
34. Akbari, O., DeKruyff, R. H. & Umetsu, D. T. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nature Immunol.* **2**, 725–731 (2001).
35. Turner, H. & Kinet, J. P. Signalling through the high-affinity IgE receptor Fc ϵ RI. *Nature* **402**, B24–B30 (1999).
36. Yamaguchi, M. *et al.* IgE enhances Fc ϵ receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: synergistic effect of IL-4 and IgE on human mast cell Fc ϵ receptor I expression and mediator release. *J. Immunol.* **162**, 5455–5465 (1999).
37. Saini, S. S. *et al.* The relationship between serum IgE and surface levels of Fc ϵ RI on human leukocytes in various diseases: correlation of expression with Fc ϵ RI on basophils but not on monocytes or eosinophils. *J. Allergy Clin. Immunol.* **106**, 514–520 (2000).
38. MacGlashan, D. Jr, Xia, H. Z., Schwartz, L. B. & Gong, J. IgE-regulated loss, not IgE-regulated synthesis, controls expression of Fc ϵ RI in human basophils. *J. Leukocyte Biol.* **70**, 207–218 (2001).
39. Kubo, S. *et al.* Drastic up-regulation of Fc ϵ RI on mast cells is induced by IgE binding through stabilization and accumulation of Fc ϵ RI on the cell surface. *J. Immunol.* **167**, 3427–3434 (2001).
40. Asai, K. *et al.* Regulation of mast-cell survival by IgE. *Immunity* **14**, 791–800 (2001).
41. Kalesnikoff, J. *et al.* Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* **14**, 801–811 (2001).
42. Katoh, N., Kraft, S., Wessendorf, J. H. & Bieber, T. The high-affinity IgE receptor (Fc ϵ RI) blocks apoptosis in normal human monocytes. *J. Clin. Invest.* **105**, 183–190 (2000).
43. Gounni, A. S. *et al.* High-affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature* **367**, 183–186 (1994).
44. Kita, H. *et al.* Does IgE bind to and activate eosinophils from patients with allergy? *J. Immunol.* **162**, 6901–6911 (1999).
45. Smith, S. J. *et al.* Blood eosinophils from atopic donors express messenger RNA for the α -, β - and γ -subunits of the high-affinity IgE receptor (Fc ϵ RI) and intracellular, but not cell surface, α -subunit protein. *J. Allergy Clin. Immunol.* **105**, 309–317 (2000).
46. Campbell, A. M. *et al.* Expression of the high-affinity receptor for IgE on bronchial epithelial cells of asthmatics. *Am. J. Respir. Cell Mol. Biol.* **19**, 92–97 (1998).
47. Hasegawa, S. *et al.* Functional expression of the high-affinity receptor for IgE (Fc ϵ RI) in human platelets and its intracellular expression in human megakaryocytes. *Blood* **93**, 2543–2551 (1999).
48. Gounni, A. S. *et al.* Human neutrophils express the high-affinity receptor for immunoglobulin E (Fc ϵ RI): role in asthma. *FASEB J.* **15**, 940–949 (2001).
49. Treter, S. & Luqman, M. Antigen-specific T-cell tolerance down-regulates mast-cell responses *in vivo*. *Cell. Immunol.* **206**, 116–124 (2000).
50. Jutel, M. *et al.* Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature* **413**, 420–425 (2001).
51. Mudde, G. C. *et al.* Allergen presentation by epidermal Langerhans cells from patients with atopic dermatitis is mediated by IgE. *Immunology* **69**, 335–341 (1990).
52. Maurer, D. *et al.* Peripheral-blood dendritic cells express Fc ϵ RI as a complex composed of Fc ϵ RI α - and Fc ϵ RI γ -chains, and can use this receptor for IgE-mediated allergen presentation. *J. Immunol.* **157**, 607–616 (1996).
53. Kraft, S., Wessendorf, J. H., Hanau, D. & Bieber, T. Regulation of the high-affinity receptor for IgE on human epidermal Langerhans cells. *J. Immunol.* **161**, 1000–1006 (1998).
54. van der Heijden, F. L., Joost van Neerven, R. J., van Katwijk, M., Bos, J. D. & Kapsenberg, M. L. Serum IgE-facilitated allergen presentation in atopic disease. *J. Immunol.* **150**, 3643–3650 (1993).
55. Haselden, B. M., Kay, A. B. & Larché, M. Immunoglobulin-E-independent major histocompatibility complex-restricted T-cell peptide epitope-induced late asthmatic reactions. *J. Exp. Med.* **189**, 1885–1894 (1999).
56. Stumbles, P. A. *et al.* Resting respiratory-tract dendritic cells preferentially stimulate T helper cell type 2 (Th2) responses and require obligatory cytokine signals for induction of Th1 immunity. *J. Exp. Med.* **188**, 2019–2031 (1998).
57. Lambrecht, B. N. *et al.* Dendritic cells induce Th2 responses to inhaled antigen leading to eosinophilic airway inflammation. *J. Clin. Invest.* **106**, 551–559 (2000).
58. Cieslewicz, G. *et al.* The late, but not early, asthmatic response is dependent on IL-5 and correlates with eosinophil infiltration. *J. Clin. Invest.* **104**, 301–308 (1999).
59. Dombrowicz, D. & Capron, M. Eosinophils, allergy and parasites. *Curr. Opin. Immunol.* **13**, 716–720 (2001).
60. Wu, W. *et al.* Eosinophils generate brominating oxidants in allergen-induced asthma. *J. Clin. Invest.* **105**, 1455–1463 (2000).
61. Bryan, S. A. *et al.* Effects of recombinant human interleukin-12 on eosinophils, airway hyperresponsiveness and the late asthmatic response. *Lancet* **356**, 2149–2153 (2000).
62. Leckie, M. J. *et al.* Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness and the late asthmatic response. *Lancet* **356**, 2144–2148 (2000).
63. Secrist, H., DeKruyff, R. H. & Umetsu, D. T. Interleukin-4 production by CD4⁺ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *J. Exp. Med.* **181**, 1081–1089 (1995).
64. Ball, T. *et al.* B-cell epitopes of the major timothy grass-pollen allergen, Phl p 1, revealed by gene fragmentation as candidates for immunotherapy. *FASEB J.* **13**, 1277–1290 (1999).
65. Bousquet, J. *et al.* WHO position paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* **53**, 1–42 (1998).
66. Durham, S. R. & Till, S. J. Immunologic changes associated with allergen immunotherapy. *J. Allergy Clin. Immunol.* **102**, 157–164 (1998).
67. Cooke, R. A., Barnard, J. H., Hebdal, S. & Stull, A. Serological evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). *J. Exp. Med.* **62**, 733–750 (1935).
68. Loveless, M. H. Immunological studies of pollinosis. I. The presence of two antibodies related to the same pollen antigen in the serum of treated hay-fever patients. *J. Immunol.* **38**, 25–50 (1940).
69. Djurup, R. & Malling, H. J. High IgG4 antibody level is associated with failure of immunotherapy with inhalant allergens. *Clin. Allergy* **17**, 459–468 (1987).
70. Flicker, S. *et al.* Conversion of grass pollen allergen-specific human IgE into a protective IgG₄ antibody. *Eur. J. Immunol.* (in the press).
71. Ball, T. *et al.* Induction of antibody responses to new B-cell epitopes indicates vaccination character of allergen immunotherapy. *Eur. J. Immunol.* **29**, 2026–2036 (1999).
72. Van Neerven, R. J. *et al.* Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4⁺ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J. Immunol.* **163**, 2944–2952 (1999).
73. Rocklin, R. E., Sheffer, A., Greiner, D. K. & Melfon, K. L. Generation of antigen-specific suppressor cells during allergy desensitization. *N. Engl. J. Med.* **302**, 1213–1219 (1980).
74. Secrist, H., Chelen, C. J., Wen, Y., Marshall, J. D. & Umetsu, D. T. Allergen immunotherapy decreases interleukin-4 production in CD4⁺ T cells from allergic individuals. *J. Exp. Med.* **178**, 2123–2130 (1993).

75. Jutel, M. *et al.* Bee-venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN- γ secretion in specific allergen-stimulated T-cell cultures. *J. Immunol.* **154**, 4187–4194 (1995).
76. McHugh, S. M., Deighton, J., Stewart, A. G., Lachmann, P. J. & Ewan, P. W. 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* **25**, 828–838 (1995).
77. Ebner, C. *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* **27**, 1007–1015 (1997).
78. Akdis, C. A. *et al.* Epitope-specific T-cell tolerance to phospholipase A2 in bee-venom immunotherapy and recovery by IL-2 and IL-15 *in vitro*. *J. Clin. Invest.* **98**, 1676–1683 (1996).
79. Akdis, C. A., Joss, A., Akdis, M. & Blaser, K. Mechanism of IL-10-induced T-cell inactivation in allergic inflammation and normal response to allergens. *Int. Arch. Allergy Immunol.* **124**, 180–182 (2001).
80. Hoyne, G. F., O'Hehir, R. E., Wraith, D. C., Thomas, W. R. & Lamb, J. R. Inhibition of T-cell and antibody responses to house dust mite allergen by inhalation of the dominant T-cell epitope in naive and sensitized mice. *J. Exp. Med.* **178**, 1783–1788 (1993).
81. Briner, T. J. *et al.* Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d 1. *Proc. Natl Acad. Sci. USA* **90**, 7608–7612 (1993).
82. Pene, J. *et al.* Immunotherapy with Fel d 1 peptides decreases IL-4 release by peripheral-blood T cells of patients allergic to cats. *J. Allergy Clin. Immunol.* **102**, 571–578 (1998).
83. Simons, F. E., Imada, M., Li, Y., Watson, W. T. & HayGlass, K. T. Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. *Int. Immunol.* **8**, 1937–1945 (1996).
84. Oldfield, W. L., Kay, A. B. & Larche, M. Allergen-derived T-cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. *J. Immunol.* **167**, 1734–1739 (2001).
85. Müller, U. *et al.* Successful immunotherapy with T-cell epitope peptides of bee-venom phospholipase A₂ induces specific T-cell anergy in patients allergic to bee venom. *J. Allergy Clin. Immunol.* **101**, 747–754 (1998).
86. Durham, S. *et al.* Long-term clinical efficacy of grass-pollen immunotherapy. *N. Engl. J. Med.* **341**, 468–475 (1999).
87. Stull, A. *et al.* Experimental and clinical studies of fresh and modified pollen extracts. *J. Allergy* **11**, 439–446 (1940).
88. Marsh, D. G., Lichtenstein, L. M. & Cambell, D. H. Studies on allergoids prepared from naturally occurring allergens. I. Assay of allergenicity and antigenicity of formalinized rye group I component. *Immunology* **18**, 449–459 (1970).
89. Lee, W. Y. & Sehon, A. H. Abrogation of reaginic antibodies with modified allergens. *Nature* **267**, 618–619 (1977).
90. van Ree, R. *et al.* False-positive skin-prick test responses to commercially available dog-dander extracts caused by contamination with house dust mite (*Dermatophagoides pteronyssinus*) allergens. *J. Allergy Clin. Immunol.* **98**, 1028–1034 (1996).
91. Valenta, R. *et al.* The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin. Exp. Allergy* **29**, 896–904 (1999).
92. Valenta, R. *et al.* Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* **253**, 557–560 (1991).
93. Movérare, R., Elfman, L., Vesterinen, E., Metso, T. & Haahtela, T. Development of new IgE reactivities to allergenic components in pollen extracts during specific immunotherapy studied with immunoblotting and the Pharmacia CAP system. *Allergy* **57**, 423–430 (2002).
94. Hiller, R. *et al.* Microarrayed allergen molecules: diagnostic gatekeepers for refined allergy treatment. *FASEB J.* 2002 Jan 14 (DOI 10.1096/fj.01–0711.fje).
95. Niederberger, V. *et al.* IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5 and Bet v 2) account for a high percentage of grass pollen-specific IgE. *J. Allergy Clin. Immunol.* **101**, 258–264 (1998).
96. Niederberger, V. *et al.* Recombinant birch pollen allergens (rBet v 1, rBet v 2) contain most of the IgE epitopes present in birch, alder, hornbeam, hazel and oak pollen. A quantitative IgE inhibition study using sera from different populations. *J. Allergy Clin. Immunol.* **102**, 579–591 (1998).
97. Kazemi-Shirazi, L. *et al.* Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy. *J. Allergy Clin. Immunol.* **105**, 116–125 (2000).
98. Valenta, R. *et al.* Genetically engineered and synthetic allergen derivatives: candidates for vaccination against type I allergy. *Biol. Chem.* **380**, 815–824 (1999).
99. Ball, T. *et al.* Isolation of an immunodominant IgE hapten from an expression cDNA library. Dissection of the allergic effector reaction. *J. Biol. Chem.* **269**, 28323–28328 (1994).
100. Smith, A. M. & Chapman, M. D. Reduction in IgE binding to allergen variants generated by site-directed mutagenesis: contribution of disulfide bonds to the antigenic structure of the major house dust mite allergen Der p 2. *Mol. Immunol.* **33**, 399–405 (1996).
101. Ferreira, F. *et al.* Dissection of immunoglobulin E and T-lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy. *J. Exp. Med.* **183**, 599–609 (1996).
102. Takai, T. *et al.* Engineering of the major house dust mite allergen Der f 2 for allergen-specific immunotherapy. *Nature Biotechnol.* **15**, 754–758 (1997).
103. Vrtala, S. *et al.* Conversion of the major birch-pollen allergen, Bet v 1, into two nonanaphylactic T-cell epitope-containing fragments: candidates for a novel form of specific immunotherapy. *J. Clin. Invest.* **99**, 1673–1681 (1997).
104. Ferreira, F. *et al.* Modulation of IgE reactivity of allergens by site-directed mutagenesis: potential use of hypoallergenic variants for immunotherapy. *FASEB J.* **12**, 231–242 (1998).
105. Schramm, G. *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.* **162**, 2406–2414 (1999).
106. Vrtala, S. *et al.* Genetic engineering of a hypoallergenic trimer of the major birch-pollen allergen Bet v 1. *FASEB J.* **15**, 2045–2047 (2001).
107. Focke, M. *et al.* Nonanaphylactic synthetic peptides derived from B-cell epitopes of the major grass-pollen allergen, Phl p 1, for allergy vaccination. *FASEB J.* **15**, 2042–2044 (2001).
108. Vrtala, S. *et al.* T-cell epitope-containing hypoallergenic recombinant fragments of the major birch-pollen allergen, Bet v 1, induce blocking antibodies. *J. Immunol.* **165**, 6653–6659 (2000).
109. Korematsu, S. *et al.* C8/119S mutation of major mite allergen Der f 2 leads to degenerate secondary structure and molecular polymerization and induces potent and exclusive Th1-cell differentiation. *J. Immunol.* **165**, 2895–2902 (2000).
110. Wiedermann, U. *et al.* Intranasal treatment with a recombinant hypoallergenic derivative of the major birch-pollen allergen Bet v 1 prevents allergic sensitization and airway inflammation in mice. *Int. Arch. Allergy Immunol.* **126**, 68–77 (2001).
111. Van Hage-Hamsten, M. *et al.* Skin-test evaluation of genetically engineered hypoallergenic derivatives of the major birch-pollen allergen, Bet v 1. Results obtained with a mix of two recombinant Bet v 1 fragments and rBet v 1 trimer in a Swedish population before the birch-pollen season. *J. Allergy Clin. Immunol.* **104**, 969–977 (1999).
112. Arquint, O. *et al.* Reduced *in vivo* allergenicity of Bet v 1d isoform, a natural component of birch pollen. *J. Allergy Clin. Immunol.* **104**, 1239–1243 (1999).
113. Pauli, G. *et al.* Clinical evaluation of genetically engineered hypoallergenic rBet v 1 derivatives by skin prick and intradermal testing: results obtained in a French population. *Clin. Exp. Allergy* **30**, 1076–1084 (2000).
114. Nopp, A. *et al.* Comparison of inflammatory responses to genetically engineered hypoallergenic derivatives of the major birch-pollen allergen Bet v 1 and to recombinant Bet v 1 wild type in skin chamber fluids collected from birch-pollen-allergic patients. *J. Allergy Clin. Immunol.* **106**, 101–109 (2000).
115. van Halteren, A. G. *et al.* Regulation of antigen-specific IgE, IgG1 and mast-cell responses to ingested allergen by mucosal tolerance induction. *J. Immunol.* **159**, 3009–3015 (1997).
116. Gabrielson, S. *et al.* Specific immunotherapy prevents increased levels of allergen-specific IL-4- and IL-13-producing cells during pollen season. *Allergy* **56**, 293–300 (2001).
117. Wilson, D. R. *et al.* Grass-pollen immunotherapy: symptomatic improvement correlates with reductions in eosinophils and IL-5 mRNA expression in the nasal mucosa during the pollen season. *J. Allergy Clin. Immunol.* **107**, 971–976 (2001).
118. Hakansson, L., Heinrich, C., Rak, S. & Venge, P. Activation of B lymphocytes during grass-pollen season. Effect of immunotherapy. *Clin. Exp. Allergy* **28**, 791–798 (1998).
119. Pierkes, M. *et al.* Decreased release of histamine and sulfidoleukotrienes by human peripheral-blood leukocytes after wasp-venom immunotherapy is partially due to induction of IL-10 and IFN- γ production of T cells. *J. Allergy Clin. Immunol.* **103**, 326–332 (1999).
120. Durham, S. *et al.* Grass-pollen immunotherapy decreases the number of mast cells in the skin. *Clin. Exp. Allergy* **29**, 1490–1496 (1999).

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Online links

DATABASES

The following terms in this article are linked online to:

Entrez: <http://www.ncbi.nlm.nih.gov/Entrez/>
bee-venom phospholipase | Bet v 1 | Fel d 1
LocusLink: <http://www.ncbi.nlm.nih.gov/LocusLink/>
FceRI | FceRII | IL-4 | IL-5 | IL-12 | IL-13
OMIM: <http://www.ncbi.nlm.nih.gov/Omim/>
asthma | atopic dermatitis

FURTHER INFORMATION

Farrp (Food Allergy Research and Resource Program) Allergen Database: <http://www.allergenonline.com>
Structural Database of Allergenic Proteins, The University of Texas Medical Branch:
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