

Review Article

Asthma therapy in the new millennium

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ABSTRACT

The application of molecular biology to the discovery of novel pathways to inhibit allergic tissue responses is becoming a reality. Particularly promising approaches are the use of vaccines and pharmacologic agents to downregulate the polarized T Helper (Th)-2 lymphocyte response through enhancement of interferon- γ production. Selective inhibition of specific mediators, such as interleukin (IL)-4, IL-5, IL-13 and eotaxin, should lead to a new class of anticytokine therapeutic agents. At the cellular level, more effective inhibition of mast cell activation and strategies to remove IgE as the triggering stimulus hold promise. With the discovery of susceptibility genes for allergic disease, the next decade is likely to witness substantial further developments in this field, with a strong focus targeted on induction mechanisms and disease prevention. If the current epidemic of allergic disease continues, then there is a strong incentive to identify those environmental factors that are responsible so that appropriate interventions can be introduced. These may include alterations to the maternal and infant diet to program the developing immune response or, in genetically susceptible individuals, the early introduction of a protective vaccine to reset the T lymphocyte balance more in favour of a Th-1 response. In this regard, the development of cytosine and guanosine nucleotide repeats and antigen-specific DNA vaccines look especially promising. In established allergic disease, the task of reversing sensitization is daunting. Safer and more efficacious allergen vaccines, whether using DNA or peptide approaches, offer the most promising

approach for fundamentally changing the allergic immune response. Patients would also greatly benefit from more effective and orally active mast cell inhibitors and small molecules that could either remove IgE or effectively interrupt its capacity to signal through its cell surface receptors. In the meantime, the epidemic of allergic disease requires urgent attention, not only in the provision of specialist centers for providing accurate diagnosis and for administering treatment (including immunotherapy), but in a clear recognition that these disorders are caused by changing environmental factors and, as a consequence, deserve attention at the level of public health.

Key words: asthma, interleukin, T lymphocytes.

INTRODUCTION

When John Bostock first described hay fever in 1819, the condition was rare and restricted to the privileged class. As we approach the turn of the century, allergy has reached epidemic proportions with almost one in two people in the developed world exhibiting an allergic response to one or other common environmental factors. Most allergic diseases are linked to atopy, the predisposition to generate the allergic antibody IgE to common environmental agents. Because IgE is able to sensitize mast cells anywhere in the body, atopic individuals frequently express disease in more than one organ. Although current treatments are able to control symptoms, allergy still causes appreciable misery. There is urgent need for novel approaches to be developed to more effectively treat allergic responses and prevent their occurrence. The present review focuses on some exciting new developments in the field based on a clearer understanding of the underlying cellular and mediator mechanisms and how the human interacts with the changing environment. Strategies based on blocking key signaling cytokines, removing IgE, inhibiting mast cells and provoking both non-specific and

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specific immune protective responses will be discussed. The introduction of molecular biology and genetics into the design of novel therapeutics over the next two decades will undoubtedly revolutionize the way these disorders are managed.

Populated-based studies have revealed large geographical differences in the prevalence of allergic disease, with countries such as the UK, Australia and New Zealand having figures 10–15-fold higher than central and eastern Europe and Asia. Although atopic disorders show strong heritability, it is differences in the environments that are likely to account for geographical variations. Also of particular concern is the rising trend in allergic disorders, both in the developed and developing world. The increase has been especially noticeable in the past two decades, predominantly affecting the young and linked to a western-type lifestyle. Based on careful epidemiologic studies, changes to maternal and infants diets, reduced exposure to antibiotics in infancy, avoidance of indoor air pollutants (especially cigarette smoke) and aeroallergens have all been suggested as steps to reverse the rising trends.¹

Current treatment options for allergic diseases are based on allergen avoidance and the use of corticosteroids to control inflammation, and antihistamines and sympathomimetics to treat symptoms. Allergen immunotherapy (desensitization) is also effective for some allergic disorders (e.g. pollenosis, insect allergy), but not others (e.g. eczema, food allergy). Central to an understanding of how susceptible (atopic) individuals develop IgE against certain environmental factors is a knowledge of how the immune system recognizes and responds to the offending agents. This involves uptake and processing of allergens, usually at a mucosal surface, by dendritic cells and subsequent presentation of a small peptide to naïve T lymphocytes.² In those destined to develop an allergic response, naïve T cells differentiate to a subtype designated T helper (Th)-2, which secretes a group of messenger proteins or cytokines responsible for switching B lymphocytes to produce IgE, and the involvement of mast cells, basophils and eosinophils. In contrast, Th-1 responses drive protective cell-mediated immunity and also inhibit Th-2 responses by their release of the cytokine interferon (IFN)- γ (Fig. 1).

INHIBITING ALLERGEN SENSITIZATION

Because most atopic disorders are acquired early in life, there is great interest in identifying those environmental

factors that lead to Th-2 polarization and the emergence of allergic disease with a view to primary prevention. In children destined to become allergic, there is impaired production of IFN- γ by their circulating T lymphocytes at birth, which persists into late childhood.³ Thus, a possible explanation for the protective effects of exposure to bacteria or their products during the period when sensitization occurs in early life is their action to increase IFN- γ production.⁴ This concept has given rise to the 'Hygiene Hypothesis', in which changes to infant diets, the early use of antibiotics and reduced exposure to bacterial products predisposes to the persistence of Th-2 responses in childhood.⁵ It follows that one approach to treating allergy would be to take advantage of the capacity of *Mycobacteria* to evoke strong interleukin (IL)-12 and, consequently, IFN- γ production as in the case of the soil saprophyte *Mycobacterium vaccae*, because it is not a human pathogen.⁶ Clinical trials of this 'vaccine' for rhinitis and asthma are in progress with early results revealing efficacy.⁷

Dendritic cells of vertebrates have evolved a defence mechanism that detects bacterial DNA through their excess of unprotected cytosine and guanosine nucleotide repeats (CpG) and offers a possible explanation for the protective action of bacteria on Th-2 responses.⁸ Synthetic DNA containing CpG motifs are recognized as danger signals by receptors on dendritic cells that serve to direct the T lymphocyte response in a Th-1 way by inducing IL-12 release, which enhances IFN- γ production (Fig. 1).⁹ Recent murine studies show that CpG DNA can be used alone or with antigen to induce Th-1 responses even in the presence of a pre-existing Th-2 response.⁹ Human studies in allergic rhinitis and asthma are being initiated.

INHIBITING IgE RESPONSES

Cross-linkage of the major affinity receptor for IgE (Fc ϵ R1) through allergen binding to cell surface IgE is the principal way in which allergens activate mast cells and basophils for mediator secretion. The Fc ϵ R1 comprises an α chain (Fc ϵ R1 α), which binds with strong affinity to IgE, two Fc ϵ R1 β chains responsible for cell signaling and a γ chain that regulates receptor signaling. Although there is debate about the precise molecular interaction between IgE and Fc ϵ R1 α , mouse monoclonal antibodies (mAbs) have been produced that inhibit IgE binding to Fc ϵ R1 α but are unable to cross-link IgE bound to mast cells or basophils and, therefore, fail to initiate mast cell activation.¹⁰ When administered intravenously to atopic

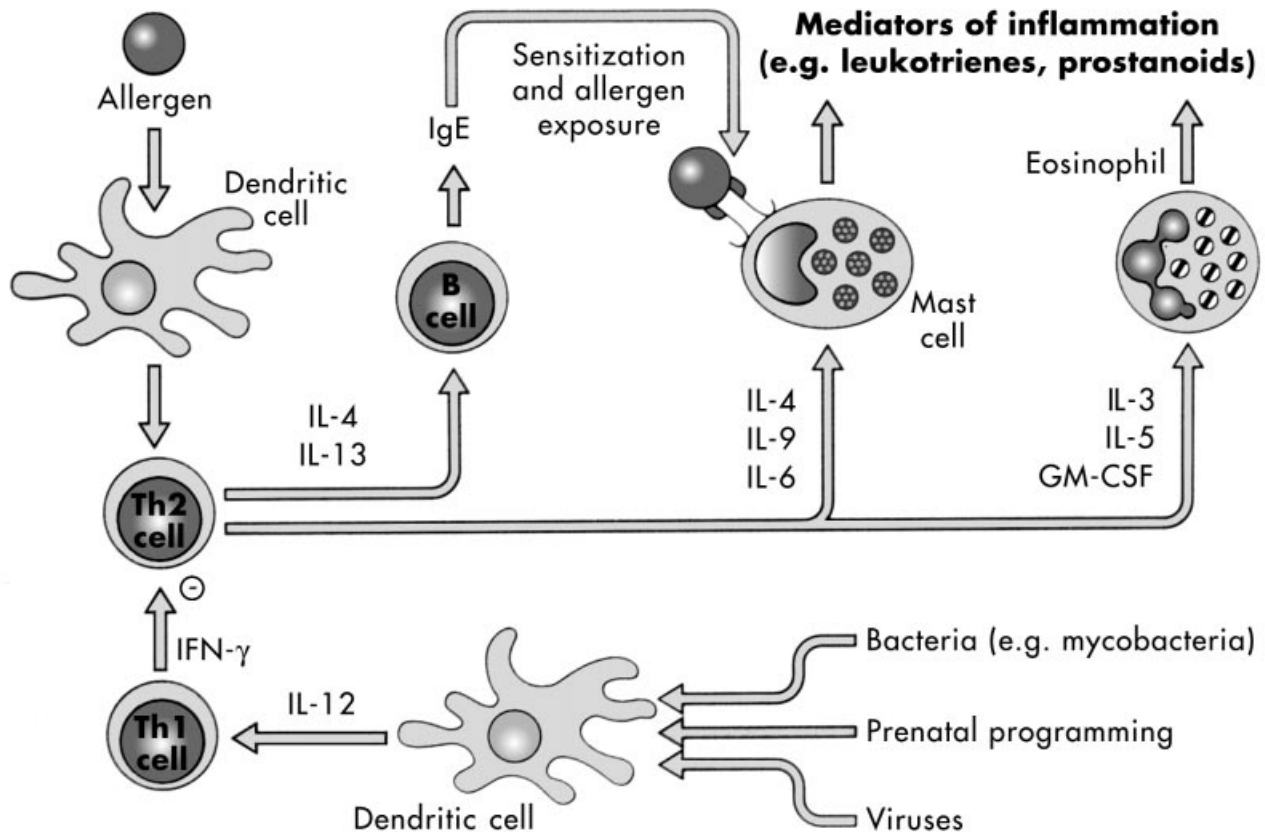


Fig. 1 The altered cytokine balance in allergic disease. IL, interleukin; Th-2, Th-1 T helper 2 and 1 cells, respectively; GM-CSF, granulocyte-macrophage colony stimulating factor.

individuals, chaemeric (CGP 51901) or fully humanized (E25) anti-IgE mAbs rapidly reduce circulating IgE to almost undetectable levels. In patients with allergic asthma, nine weekly injections of E-25 mAb almost abolished the early (mast cell mediated) and late (inflammation mediated) bronchoconstriction to inhaled allergen.¹⁰ The mAb E-25 is also effective in controlling clinical asthma, reducing exacerbations and oral corticosteroid requirement by 50% as well as improving baseline lung function and reducing bronchodilator use.¹⁰ Anti-IgE therapy has also proven to be efficacious in allergic rhinitis.¹¹ Clinical trials are in progress to assess its efficacy in severe asthma.

In 1990, a decapeptide within IgE was shown to elicit antibodies that blocked anaphylactic histamine release in rabbits.¹² One report has shown that this 'vaccine' is efficacious against food allergy in humans,¹³ but a further clinical trial has failed to confirm this. Nevertheless, other peptide sequences of the FcεR1-binding regions of IgE offer great promise as anti-allergic vaccines¹⁴ and, while some concern had been expressed that removal

of IgE will reduce protection against parasites, animal studies have so far failed to show this.

INHIBITING MEDIATOR RELEASE OR THEIR EFFECTS

Although sodium cromoglycate has been regarded as the archetypal anti-allergy drug, its relatively low efficacy for inhibiting mast cells has been problematic. The identification of proteins that contain immunoreceptor tyrosine-based inhibitory motifs (ITIM; e.g. mast cell-associated function antigen (MAFA), gp 49B1 and FcγRIIB receptors) provide new targets for mast cell inhibition.¹⁵ These cell-surface proteins associate with FcεR1 within membrane 'rafts' and potentially inhibit mast cell activation by recruiting enzymes to dephosphorylate FcεR1β and FcεR1γ chains to interrupt IgE signaling.

The clear involvement of inflammatory mediators, such as histamine, prostaglandins and leukotrienes, which interact in allergic responses, has stimulated the development of drugs that either inhibit their formation

or selectively block their effects. An alternative approach is to neutralize mediators once they have been released, similar to the binding and inactivation of cytokines by soluble receptors. In order to minimize tissue reactions, hard ticks have evolved proteins in their saliva that do just this. *Rhipicephalus appendiculatus* is a tick that secretes saliva in its three histamine-binding proteins (Ra-HBP). Histamine-binding protein-2 (RaHP2) has two internal histamine binding pockets and a folding pattern not dissimilar to other lipocalins that exist to bind small molecular weight chemicals.¹⁶ The Ra-HBP1, Ra-HBP2 and Ra-HBP3 have been cloned and the recombinant proteins expressed. *In vitro*, they inhibit mast cell mediator induced-contraction of sensitized tissues and one such molecule is currently in clinical trials. It is highly likely that other selective mediator inhibitors are awaiting discovery in tick saliva, the activity of which could be harnessed to create novel anti-allergic agents.¹⁶

The recent discovery that many allergens possess enzymatic activities raises the issue as to whether such biological properties create some advantage for the molecules in becoming sensitizing agents. The cysteine protease of the dust mite allergen *Der P₁* can cleave human low-affinity IgE receptor (FcεR2, CD23; involved in IgE regulation), IL-2 receptor (involved in T-cell activation) and occludin (a key protein in epithelial tight junctions), which could lead to an increase in local IgE synthesis, Th-2 polarization and penetration of allergen into mucosal tissues.¹⁷ The importance of proteolytic enzymes in enhancing sensitization is now being realized as is the potential benefit from blocking these activities with selective inhibitors, such as PTL 11028.¹⁸

TARGETING PRO-ALLERGIC CYTOKINES

Interleukin-4 and its homologue IL-13 are pivotal in regulating the allergic phenotype (Fig. 1) and produce their cellular effects through the signal transducer and activator of transcription (STAT)-6.¹⁹ Interleukin-4 (but not its homologue IL-13) is essential for maintaining the Th-2 T cell phenotype, although for many of the other functions either cytokine is effective (Table 1). If the genes for IL-4, IL-13 or STAT-6 are deleted in mice, IgE production and antigen-induced airway inflammation are severely attenuated. In the double IL-13/IL-4 knock-out mouse IgE production is abolished. While humanized blocking mAbs against IL-4 have been developed, the preparation of a recombinant soluble form of the human IL-4 receptor α chain without its transmembrane

and cytoplasmic regions (sIL-4r) has shown efficacy in a mouse model of immediate hypersensitivity²⁰ and, more recently, in atopic asthma.²¹ Interleukin-13 is produced in large amounts by asthmatic airways and has been incriminated as an important cytokine in mouse models of asthma. Not only is it involved in IgE regulation, but it is also profibrogenic and induces mucus metaplasia of the epithelium.^{22,23} Although IL-13 binds to one of the two subtypes of IL-4r (IL-4 α /IL-13 α), it will not be recognized by soluble IL-4r. Thus, an alternative approach to inhibit both IL-4 and IL-13 functions has been to use a double mutant of IL-4 (Arg-121-Asp and Tyr-124-Asp; BAY 16-9996) which is an antagonist.²⁴ Administration of the IL-4 double mutant to mice completely prevented the allergic antibody response as well as cutaneous anaphylactic responses on exposure to sensitizing antigen.²⁵ In IL-4- or IL-13-responsive cells, STAT-6 itself is also a selective target, inhibition of which will prevent signaling by both IL-4 and IL-13.¹⁹

Interleukin-5 is a Th-2 cytokine that is considered essential for the recruitment of eosinophils in allergic inflammatory responses (Fig. 1; Table 2). In animals, administration of an anti-IL-5 blocking mAb inhibits eosinophil recruitment from the bone marrow into tissues, resulting in ablation of the late-phase response to inhaled antigen.²⁶ Recently, a humanized anti-IL-5 blocking mAb (SB-240563) has become available, which, when administered to atopic asthmatic subjects as a single intra-venous dose, reduced both blood and sputum eosinophils but, surprisingly, failed to inhibit either the allergen-provoked late-phase bronchoconstriction or

Table 1 Pro-allergic functions of interleukin-4 and -13

	Interleukin-4	Interleukin-13
T helper-2 cell polarization	+	–
B cell IgE switching	+	+
Upregulation of vascular cell adhesion molecule	+	+
Upregulation of mucus genes	+	+
Increased fibroblast proliferation and collagen production	+	+
Decreased monocyte functions	+	+

Table 2 Proallergic functions of interleukin-5

Maturation of eosinophils
Priming of eosinophils for enhanced mediator secretion
Chemoattraction of eosinophils
Protection of eosinophils from apoptosis

airway hyperresponsiveness.²⁷ This result questions the association between IL-5 secretion and induced eosinophil recruitment as being obligatory for the human late-phase allergic response, but does not reduce interest in this approach for treating chronic eosinophil-mediated disorders that rely on IL-5.²⁸

The recent discovery that a cluster of cytokines belonging to the CC chemokine family, which includes three eotaxins, MCP-1, -3 and -4 and RANTES, are powerful eosinophil chemoattractants using a common cell surface receptor (CCR-3), opens up another important target for intervening in allergic disease.²⁸ A mAb directed against CCR-3 blocks eosinophil migration.²⁹ Human mAbs against eotaxin itself are being developed, although, on account of chemokine redundancy, this will probably be a less effective strategy than developing selective antagonists against the CCR-3 receptor itself. Using bacteriophage expression libraries and combinatorial chemistry, highly selective peptide and non-peptide CCR-3 receptor antagonists are being discovered. Recent research indicates that CCR-3 and IL-5r ligands work co-operatively to recruit eosinophils into target tissues.²⁸

ALLERGEN-SPECIFIC IMMUNOTHERAPY

The aim of immunotherapy is to vaccinate with allergen or its derivatives to produce selective inhibition of allergen-specific responses. While undoubtedly efficacious in a number of serious allergic disorders (e.g. insect or penicillin anaphylaxis), care has to be exercised in order not to provoke local and systemic anaphylactic reactions. Indeed, in some countries, such adverse reactions have limited the uptake of immunotherapy as a therapeutic option. In an attempt to circumvent this, many allergens have been cloned and mutated to reduce interactions with IgE without changing the epitopes responsible for the development of T cell tolerance. Strategies are also in place to develop peptides that will inhibit T cell responses to allergens but fail to trigger IgE-mediated mast cell responses. Because immunotherapy is thought to be protective, in part, by inducing a Th-1 IFN- γ (Fig. 1),³⁰ attempts to enhance the efficacy of allergen-immunotherapy by generating allergen peptides in *M. vaccae* are being actively pursued.

A further breakthrough to intervene in allergy has been the use of antigen-selective DNA vaccines to induce allergen synthesis and a powerful protective immune response in the host. Leong *et al.* have cloned a

major peanut allergen into a synthetic vector in which the DNA is protected from digestion by chitosan, a naturally occurring polysaccharide used for the controlled intestinal delivery of many pharmaceutical agents.³¹ After ingestion, nanoparticles of the polysaccharide adhere to intestinal epithelial cells to facilitate the uptake of peanut-specific DNA. When administered orally to mice, this 'vaccine' protected animals against both sensitization and anaphylaxis upon subsequent peanut challenge. A critical question to answer is whether DNA vaccines can produce tolerance if given to an already sensitized subject. It is encouraging that at least one controlled study has shown that sublingual administration of a grass pollen extract is beneficial in established seasonal allergic rhinitis.³²

REFERENCES

- 1 Peat JK, Li J. Reversing the trend: Reducing the prevalence of asthma. *J. Allergy Clin. Immunol.* 1999; **103**: 1–10.
- 2 Palmer EM, van Seventer GA. Human T helper cell differentiation is regulated by the combined action of cytokines and accessory cell-dependent co-stimulatory signals. *J. Immunol.* 1997; **158**: 2654–62.
- 3 Jung T, Moessner R, Kieckhoff K, Heidrich S, Neumann C. Mechanisms of deficient interferon- γ production in atopic diseases. *Clin. Exp. Allergy* 1999; **29**: 912–19.
- 4 Shirikawa T, Enomoto T, Shimazu SI, Hopkin JM. The inverse association between tuberculin response and atopic disorder. *Science* 1997; **275**: 77–9.
- 5 Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. *Lancet* 1999; **354** (Suppl. II): S11–12–15.
- 6 Wang C-C, Rook AW. Inhibition of an established allergic response to ovalbumin in BALB/c mice by killed *Mycobacterium vaccae*. *Immunology* 1998; **93**: 307–13.
- 7 Hopkin JM, Shaldan S, Ferry B *et al.* Mycobacterial immunisation in grass pollen immunisation and rhinitis. *Thorax* 1998; **53** (Suppl. 4): A16 (Abstract).
- 8 Kreig AM. An innate immune defence mechanism based on the recognition of CpG motifs in microbial DNA. *J. Lab. Clin. Med.* 1996; **128**: 128–33.
- 9 Chu RS, Targoni OS, Krieg AM, Lehmann PV, Harding CV. CpG oligodeoxynucleotides act as adjuvants that switch on T-helper 1 (Th-1) immunity. *J. Exp. Med.* 1997; **186**: 1623–8.
- 10 Patalano F. Injection of anti-IgE antibodies will suppress IgE and allergic symptoms. *Allergy* 1999; **54**: 103–10.
- 11 Casale T, Bernstein IL, Busse WW *et al.* Use of an anti-IgE humanised monoclonal antibody in ragweed-induced allergic rhinitis. *J. Allergy Clin. Immunol.* 1997; **100**: 110–21.
- 12 Stanworth DR, Jones VM, Lavin IV, Naggar S. Allergy treatment with a peptide vaccine. *Lancet* 1990; **336**: 1279–81.

- 13 Romanski B, Bartuzi Z, Stanworth DR. Assessment of a novel anti-allergy vaccine in subjects with severe food allergy and at risk of anaphylaxis to food. *Int. Rev. Allergol. Clin. Immunol.* 1998; **4**: 164–73.
- 14 Rudolf MP, Vogel M, Kricek F *et al.* Epitope specific antibody response to IgE by mimotope immunisation. *J. Immunol.* 1998; **160**: 3315–21.
- 15 Daëron M. ITIM-bearing negative coreceptors. *Immunologist* 1997; **5**: 79–85.
- 16 Paesen GC, Adams PL, Harlos K, Nuttall PA, Stuart DI. Tick histamine-binding proteins: Isolation, cloning and three-dimensional structure. *Mol. Cell* 1999; **3**: 861–71.
- 17 Sharma S, Lewis SA, Oakley IG *et al.* Airway hyperreactivity and inflammation induced by intratracheal exposure to house dust mite extract in rats. *Am. J. Respir. Crit. Care Med.* 1999; **159**: A231 (Abstract).
- 18 Billson J, Clark J, Conway SP *et al.* The design and synthesis of inhibitors of the cysteine protease Der P₁. *Bioorg. Chem. Med. Lett.* 1998; **8**: 993–9.
- 19 Foster PS. STAT-6: An intracellular target for the inhibition of allergic disease. *Clin. Exp. Allergy* 1999; **29**: 12–15.
- 20 Renz H, Bradley K, Enssel K, Loader JE, Larsen GL, Gelfand EW. Prevention of the development of immediate hypersensitivity and airway hyperresponsiveness *in vivo* with soluble IL-4 receptors. *Int. Arch. Allergy Immunol.* 1996; **106**: 167–76.
- 21 Borish LC, Nelson HS, Lanz M *et al.* Phase I/II study of interleukin-4 receptor (IL-4r) in moderate asthma. *J. Allergy Clin. Immunol.* 1998; **101**: S8–9.
- 22 Grunig G, Warnock M, Wakil AE *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998; **282**: 2261–3.
- 23 Zhu Z, Homer RJ, Wang Z *et al.* Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J. Clin. Invest.* 1999; **103**: 779–88.
- 24 Tony H-P, Shen B-J, Reusch P, Sebald W. Design of human interleukin-4 antagonists to inhibiting interleukin-4 dependent and interleukin-13-dependent responses in T cells and B cells with high efficiency. *Eur. J. Biochem.* 1994; **225**: 659–65.
- 25 Grunewald SM, Werthemann A, Schnarr B *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 response to allergen and development of allergic symptoms *in vivo*. *J. Immunol.* 1998; **160**: 4004–9.
- 26 Danzig M, Cuss F. Inhibition of interleukin-5 with a monoclonal antibody attenuates allergic inflammation. *Allergy* 1997; **52**: 787–94.
- 27 Leckie MJ, ten Brinke A, Lordan J. SB 240563, a humanised anti-IL-5 monoclonal antibody: Initial single, dose safety and activity in patients with asthma. *Am. J. Respir. Crit. Care Med.* 1999; **159**: A624 (Abstract).
- 28 Teran C. Chemokines and IL-5: Major players of eosinophil recruitment in asthma. *Clin. Exp. Allergy* 1999; **29**: 287–90.
- 29 Heath H, Qin S, Rao P *et al.* The importance of CCR-3 demonstrated using an antagonistic monoclonal antibody. *J. Clin. Invest.* 1997; **99**: 178–84.
- 30 Durham S, Till SJ. Immunologic changes associated with allergen immunotherapy. *J. Allergy Clin. Immunol.* 1998; **102**: 157–64.
- 31 Roy K, Mao HQ, Haeng SK, Leong KW. Oral gene delivery with chitosan-DNA nanoparticles generate immunological protection in a murine model of peanut allergy. *Nat. Med.* 1999; **5**: 387–91.
- 32 Clavel R, Bousquet J, André C. Clinical efficacy of sublingual-swallowed immunotherapy. A double blind placebo-controlled trial of a standardised five grass pollen extract in rhinitis. *Allergy* 1998; **53**: 493–8.