

The Journal of Laryngology & Otology
December 2003, Vol. **117**, pp. 940–945

Historical Articles

Allergen immunotherapy in ENT: historical perspective

D. S. KIM, A. B. DRAKE-LEE

Abstract

The origins of immunology and allergy are founded upon the early 19th century microbiological studies of Jenner and Pasteur. It was discovered that the immune system could cause harm. The subspecialty of allergy began with the coining of the term by Von Pirquet in 1906 to describe disorders resulting from hyper-reaction to normally innocuous environmental agents.

Understanding the scientific basis of the immune system and allergy allowed Noon and Freeman, and later Cooke, to develop allergen immunotherapy. Initially the technique was crude, but with the subsequent key discovery of IgE, more accurate methods of diagnosis (such as the radioallergosorbent test (RAST)) and treatment ensued. The efficacy of specific immunotherapy has been demonstrated by many double-blind trials culminating in the WHO position paper.

DNA recombinant technology has provided detailed molecular understanding of allergic disorders, which has resulted in several novel methods of immunotherapy that are potentially safer and more effective. Use of recombinant allergens, T-cell peptides, DNA vaccination with CpG motifs or plasmid vectors and anti-IgE strategies with monoclonal antibodies are showing promise.

Key words: History of Medicine; Allergy; Specific Immunotherapy; Otolaryngology

Origins of immunology and allergy

In the late 18th century, an English country doctor in Gloucestershire, named Edward Jenner, made his historical observations with the ‘variolation’ technique, leading to the development of the cowpox-smallpox vaccination. His observations introduced the concept of an immune system and the feasibility to use it artificially for human benefit against infections.

Louis Pasteur, having inherited this concept of artificial immunity, also became interested in the study of the body’s response to infection and how this could be manipulated. His work between 1879–1881 led to the discovery of the first three attenuated vaccines against chicken cholera, rabies and anthrax and has placed him as one of the pioneering fathers of modern immunology.

Meanwhile, isolation of the diphtheria toxin by Roux and Yersin, and serum bactericidins by Nuttall, were important steps in revealing the antigen and antibody components of the immune system. This led naturally to the development of diphtheria-specific antitoxins in 1890 by Behring and Kitasato. Establishment of the Pasteur Institute in 1888

represented the official recognition that a study in immunity was worthwhile.

Later it became apparent, during experiments with vaccines and antitoxins, that the immune system could be harmful, as well as protective. In 1890, Koch noted an adverse skin reaction to the tubercle bacillus during his experiments, and in 1893 Behring described these events as a phenomenon of ‘hypersensitivity’ to the agents used. The term ‘anaphylaxis’ was later coined by Portier and Richet in 1902 to denote the frequently observed lethal state of shock induced by a second injection of antigen.

Shortly after, the term ‘allergy’ was introduced by Clarens Von Pirquet in 1906. Von Pirquet was born in Hirschstetten near Vienna in 1874. He became the director of the Universitäts Kinderklinik and Professor of Paediatrics in Vienna in 1911, but throughout his career he had a special interest in the field of immunology. The introduction of the term ‘allergy’ was the direct result of his study of skin reactions to cowpox vaccine and attempt to explain the ‘early reaction’ at 24 hours after vaccination. The terms ‘immunity’ and ‘hypersensitivity’ already in usage seemed inadequate. Immunity implied a protective response, whereas hypersensitivity described a severe systemic response. Not all adverse

From the Department of Otolaryngology, Queen Elizabeth Hospital, Birmingham, UK.
Accepted for publication: 14 July 2003.

immunological reactions were so dramatic and thus he coined allergy to denote a 'deviation from the original state' of an organism due to its contact with an organic agent, whether live or inanimate. This original broad definition has been altered and manipulated and today the definition of allergy is often used synonymously with the more specific IgE-mediated reactions. There are still those who hold on to the broader meaning and apply allergy to all the hypersensitivity responses (as described by Gell and Coomb's classification of allergic reactions in 1968). Sadly, Von Pirquet took his own life at the age of 55 in 1929.

The beginnings of allergen immunotherapy

The syndrome of symptoms we now call 'allergy' was first described by Leonardo Bottallo in Europe in the mid-16th century and further detailed in 1819, by John Bostock in London, who described a classic case of hay fever based on his own symptoms. This descriptive paper is considered a classic, written in a time when most medical knowledge was based purely on clinical description of the disease process. The allergic nature of the syndrome had not yet been conceptualized. It was only with the elucidation of its aetiology, did the immunological basis of the symptoms gradually materialize. Morrill Wyman, an American physician first identified ragweed as a cause of the then so-called 'autumnal catarrh' and published his report in 1872. Charles Blackely, a year later, published his observation that hay fever was caused by grass pollen and described his skin testing for pollen.¹ He had recognized a relationship between the wheal and flare response produced by applying grass pollen to his own abraded forearm skin and his respiratory symptoms during the grass pollen season.

Following these discoveries, many experimented with pollen extracts in an attempt to 'immunize' those suffering from rhinitis and asthma, as Jenner and Pasteur had done against bacterial infections, a century earlier. Leonard Noon trained in infectious diseases and whilst working in the prestigious Wright-Fleming Institute, set about inducing the body to produce antitoxins to pollen, as Wright was doing with tetanus. In 1910, working with Freeman at St Mary's Hospital, London, he identified those susceptible by observing reactions to pollen extracts on abraded skin and introduced therapy with subcutaneous injections of these extracts at dilute levels, gradually raising the strength of the solution until the patient experienced symptom relief. Noon and Freeman's experiments represented the beginnings of 'desensitizing immunotherapy'.^{2,3} The concept of blocking antibodies was still far in the future. However, clinically, the relationship between skin sensitivity, specific offenders, and the therapeutic effects of treating these disorders with escalating doses of the offending agent, had now been established. The first controlled trial of allergen immunotherapy was to be performed much later in 1954 by Frankland⁴ (also at St Mary's Hospital), which demonstrated good clinical efficacy.

The problem of standardizing the allergen extract dose in order to perform reproducible and safe treatment was a particular problem to all. No uniform means existed either of producing allergen extracts, or of comparing the allergenic potencies. Crude extraction of allergens often led, in the early stages, to either fatal allergic reactions or erratic study results. As a result, there were many attempts to devise dose standardization methods. Noon made the first attempt at standardizing allergen doses and established the 'Noon unit' based on allergen weight, but the more scientific, yet still simple, crude and flawed, method of quantifying the protein nitrogen content, as devised by Cooke, became the most widely accepted. The situation is still little better than it was over 50 years ago. Even to this day there does not exist a sound method.

Robert A. Cooke, born in 1880 in New York, became the chief American proponent of the field of allergy as early as 1910, and introduced immunotherapy to the US, having adopted Noon and Freeman's theories. In 1916 he wrote a landmark paper on human allergies, summarizing 621 cases of 'human protein sensitization' over a five-year period and also described the inheritance characteristics of atopy.⁵ In the process, Cooke established his own method of allergen dose standardization by nitrogen content as determined by the Kjeldahl method. This eventually became the 'protein nitrogen unit' still in use today. Cooke became the president of the American Society for the Study of Allergy and Allied Conditions in 1924, which later merged into the American Academy of Allergy, Asthma and Immunology (AAAAI). Although a general allergist, Cooke was particularly interested in allergic disorders in the head and neck region and in 1935, presented his results on grass pollen immunotherapy for the treatment of hayfever: one of the greatest contributions to the field of allergy and immunotherapy.⁶ He described the concept of 'blocking antibodies' and in doing so provided one of the earliest explanations for the clinical improvement with immunotherapy. We now know this blocking antibody to be IgG4. Finally, Cooke introduced intradermal skin testing.

The basic principles of immunotherapy began by Noon, was developed further by Cooke and his method remains the basis for modern immunotherapy today. Cooke's technique involves the administration of arbitrary initial small doses, which is slowly increased in a step-wise manner over weeks and months until a maximum tolerated dose is achieved, then this maintenance dose is then continued for years. Unfortunately, the initial 'small' antigenic dosage varied greatly among therapists. Some used far too high doses causing frequent anaphylaxis whilst others used inadequate doses resulting in prolonged and unsatisfactory treatments.

Otolaryngic allergy in USA: Hansel-Rinkel School of immunotherapy

A major but controversial contribution to the field of otolaryngic allergy and the birth of a new allergen immunotherapy methodology was due to an

American otolaryngologist French Keller Hansel. Hansel was born in 1893, in Fidelity, Illinois and after graduating from the St Louis School of Medicine, he became a Fellow in Otolaryngology at the Mayo Clinic, Rochester, USA. His histological examination of surgical specimens highlighted the eosinophilic infiltration of the nasal and sinus mucosae. Hansel concluded that allergy and not infection, as widely thought, was the cause of this common condition.

Later, working as a faculty member of the Otolaryngology Department at the University of Washington, St Louis, USA, Hansel published his landmark paper *Allergy as etiologic factor in paranasal sinus diseases*.⁷ Based on his discovery, he proposed a more conservative approach to the then prevalent surgical management of allergic sinus diseases. He had also observed that the mould and dust extract concentrations of 1:10 and 1:100 dilutions widely used for immunotherapy at the time, caused unfavourable reactions. When he reduced the dilutions to 1:1,000 and even to 1:100,000, the results improved. Furthermore, he found that once the effective low dose had been established by skin titration, the patient did better on that same dose than the incremental dose regimen then widely practiced. This became Hansel's 'minute optimal dose immunotherapy' technique:⁸ a concept not accepted by the general allergists. Due to internal political problems, he retired early, and established the Hansel foundation in 1947, to continue his work independently and train those interested in his newly adapted method of immunotherapy.

Herbert John Rinkel, a physician and a general allergist, born in 1896 in Edwardville, just outside St Louis, was impressed by Hansel's results with the optimum dosage technique. Rinkel established a working relationship with Hansel and later evolved a new method of dilutional skin titration to arrive at the optimum dose, the 'skin endpoint titration' (SET).⁹

Hansel-Rinkel's method of immunotherapy and SET has been consistently rejected by the traditional allergist camp and remains highly controversial. Numerous double-blind randomized studies have shown SET and Rinkel's method to be ineffective.¹⁰⁻¹⁴ In a series of studies by Van Metre *et al.*^{10,13,14} they demonstrated the traditional immunotherapy technique as innovated by Cooke, to offer significant improvements in symptoms and immunological markers whereas Rinkel's method proved to be no more effective than placebo treatments in treating ragweed-pollen allergy. Furthermore, those patients who gained little benefit from Rinkel's methods were shown to respond well to subsequent traditional immunotherapy treatment.¹³ These data were further reinforced by a multi-centre double-blind study comparing Rinkel's method and histamine-placebo treatment, which demonstrated no difference in efficacy.¹¹ The WHO and other major allergy societies met in Geneva in 1997 and published the WHO immunotherapy position paper.¹⁵ They did not

recommend Rinkel's method of allergen immunotherapy in light of these consistent refuting studies. In the USA many otolaryngologists and some general allergists continue to use this technique despite strong evidence refuting its efficacy.

IgE discovery and modern laboratory based immunotherapy

A major advance in allergy studies came in 1921 when Prausnitz and Kustner described a skin sensitizing factor that can be transferred to the skin of a non-sensitized individual and found to confer sensitivity of the original antigen.¹⁶ They developed the 'PK test' to identify the presence of this factor in serum. Coca and Cooke in 1923 coined the term 'reagin' to label this key factor, but it took over 40 years of intensive efforts to identify this reagin as an antibody by two groups working independently in the mid-sixties, one in the USA and one in Sweden. Teruko and Kimishige Ishzaka in Denver, USA identified this factor which we now call IgE in 1966.¹⁷ Concurrently, a Swedish group of Hans Bennich and S G O Johansson also identified a strange protein in a multiple myeloma patient that was typically elevated in allergic patients.¹⁸ It was later decided by the WHO in 1968, that the two new proteins were the same. The identification of IgE was a key step in the diagnosis and treatment of allergy.

Shortly after, *in vitro* diagnostic tests for the detection of IgE were rapidly forthcoming. Only a year after the discovery of IgE, Wide, Bennich and Johansson developed the first in-vitro test known as RAST (radioallergosorbant test)¹⁹ and commercially marketed it as 'Phadebas-RAST' in 1970. This test was able to identify and quantify the amount of IgE in a patient's serum specific for the antigen in the same manner the skin test identified the 'reagin' in the skin. Unfortunately, the first assay showed very low sensitivity and thus had little clinical value. However, during the 1970s Richard Fadal and Donald Nalebuff (an otolaryngologist) modified the initial test and its scoring structure, increasing the sensitivity sufficiently to be practical for clinical use.²⁰ The 'Modified RAST' test (1977) rapidly became comparable with SET. Numerous studies have shown RAST to correlate reliably with allergic disease history, titrated skin tests and initial immunotherapy doses. An explosion of new *in vitro* assays for allergy diagnosis followed. The Pharmacia CAP system offers a fully automated and compact office-based system, and is now the most widely used IgE assay in US, Europe and Far East.

Clinical trials

The first controlled clinical trials evaluating the efficacy of immunotherapy was performed by Franklin and Augustin in 1954 using crude grass pollen extracts to effectively alleviate summer hay fever and asthma, and then by Lowell in 1965 on ragweed hayfever immunotherapy.^{4,21}

Since then, subcutaneous specific immunotherapy (SIT) has been widely evaluated and has been shown to be effective in various allergic conditions. The role of allergen immunotherapy and guidelines for its use has been summarized in a recent WHO position paper.¹⁵ Double-blind studies have shown SIT to be highly effective in allergic rhinitis when induced by grass pollen,²²⁻²⁴ ragweed pollen,²⁵ various other pollens²⁶ and house dust mite.^{27,28} Other studies have demonstrated improvement in bronchial symptoms during cat specific immunotherapy but nasal symptoms were not specifically monitored.^{29,30} However, it is likely that it is also effective for rhinitis.

A total of 43 double-blind studies compared specific immunotherapy versus placebo treatment and showed a mean reduction in symptom of 45 per cent with immunotherapy compared with placebo.²⁶ In addition to the demonstrated intra-treatment efficacy, more recent investigations have demonstrated long-term benefits after discontinuation of immunotherapy. Durham *et al.*³¹ demonstrated a prolonged clinical benefit and evidence of decreased immunologic reactivity for at least three years after cessation of grass pollen immunotherapy for rhinitis.

Today and hope for future

There is convincing evidence of a so-called 'Th1-Th2' concept: that a selective subpopulation of T-helper (Th) cells plays a key role in the orchestration of the inflammatory responses to inhaled allergens in rhinitis and asthma.³² Analysis of blood, mucosa biopsies and secretions revealed predominant Th-2 activation associated with IL-4, IL-5 and IL-13 secretion but not IL-2 or INF-gamma. IL-13 and IL-4 is known to regulate IgE production, which is fundamental in allergy disorders. It is believed Th2-mediated immunity exists in fetal immunity and normally this gradually changes to a Th-1-mediated immunity. However in atopic individuals, this may fail leading to a persistence of high Th-2 responses upon allergen exposure.³² The inhibition of a Th-2 response may antagonize the harmful cytokine production; studies with monoclonal antibodies to IL-4 and IL-5 have shown beneficial effects in animal models.^{33,34}

The mechanism of specific immunotherapy is complex and still unclear. Early investigation focused on immunoglobulin levels and in particular the so-called 'blocking' IgG³⁵ as initially suggested by Cooke. Subsequent studies have shown no association between immunoglobulin levels and clinical benefit. More recent data suggest immunotherapy acts by modifying the T-cell responses to allergens either by immune-shift in up-regulation of Th1 responses (increased IL-12 and IgG response)³⁶ or by decreasing Th2 responses (T-cell 'anergy' resulting in decrease in IgE, IL-4/5 and pro-inflammatory cytokines)³⁷ or both. Increase in IL-10 secretion seems to be important and has been shown to confer allergen-specific hyporesponsiveness and to suppress allergen-specific Th2

response.³⁸ Inflammatory cell recruitment, activation and cytokine release is reduced in successful immunotherapy.³⁹

Specific immunotherapy carries risks including fatal systemic anaphylaxis. With the aid of recent strides in molecular biology several novel methods of immunotherapy are being evaluated potentially offering improved safety and efficacy.

Recombinant allergens

Allergen isoforms with a reduced capacity to bind IgE have been isolated and found to consist of amino acid deletions or substitutions at key sites that affect the IgE binding site.⁴⁰ There has been detailed analysis and mapping of IgE-binding epitopes using DNA recombinant technology for a number of major allergens. Several groups^{41,42} have exploited these aspects and created by *in vitro* mutagenesis recombinant allergen proteins with substantially reduced IgE-binding capacity. Such hypoallergenic isoforms may minimize the risk of anaphylaxis. Allergen engineering also offers the prospect of purified standardized allergens reagents as opposed to the more crude and variable extracts used presently.

T-cell peptide immunotherapy

Much attention has focused on functional inactivation of T-cell responses leading to 'tolerance' to allergens in view of the prominent role of these cells in allergy pathobiology. In murine models, injection of small peptides produced T-cell anergy (hyporesponsive).⁴³ The precise mechanism of this effect remains elusive. There is also evidence for peptide-induced non-responsiveness of human CD4 T-cells in *in vitro* experiments.⁴⁴ This attractive idea of using allergen-derived peptides that lack IgE reactivity (and thus lacking immediate type reactivity) due to their small size, to induce tolerance has been evaluated in several clinical trials. Norman *et al.*⁴⁵ demonstrated limited benefit in those suffering from cat allergy and a multi-centre study reported only modest improvements in lung functions after peptide therapy.⁴⁶ Unfortunately in these early trials treatment was associated with significant incidences of adverse reactions. A more recent randomized controlled trial using Fel d1 peptide in patients suffering cat allergy demonstrated some benefit in both symptoms and immunological parameters in those treated but due partly to low sample number the observed data was not statistically significant when compared to the placebo group.⁴⁷

DNA vaccines

In contrast to the strategies employing whole proteins or peptides to modify the immune response to allergens, recent interest has focused on direct immunization with DNA encoding the allergen protein. Several variations on the theme of DNA vaccination have been investigated.

- Immunology and allergy were founded upon the early 19th century microbiological studies of Jenner and Pasteur
- The subspecialty of allergy began with the coining of the term by Von Pirquet in 1906 to describe disorders resulting from hyper-reaction to normally innocuous environmental agents
- Understanding of the scientific basis of the immune system and allergy led to the development of allergen immunotherapy
- DNA recombinant technology has resulted in several novel methods of immunotherapy that are potentially safer and more effective

Oligonucleotide sequences containing CpG motifs are found in bacterial DNA. When injected in mice, they have been shown to induce Th1 immune responses by stimulating the production of Th1-enhancing cytokines such as IL-12, IFN-gamma and TNF-gamma.⁴⁸ Another approach has been to vaccinate with plasmid vectors containing allergen-encoding DNA. In various animal models, this technique has shown to markedly reduce Th2 responses, enhance Th1 responses and effectively suppress the allergic response.⁴⁹ However the majority of the data collected in this area has come from animal models and needs caution when extrapolating to the human model.

Anti-IgE

Other therapeutic approaches under evaluation include ways to reduce IgE function. Treatment with recombinant monoclonal antibodies against IgE has been shown to eliminate IgE and significantly reduce symptoms of allergic rhinitis⁵⁰ and steroid need in chronic asthmatics.⁵¹ Another strategy involves the application of 'mimotope' technology. Mimotopes are peptides that mimic IgE binding sites of the allergen. They induce blocking IgG antibodies against the IgE epitopes.⁵² They mimic the binding sites for only one species of IgE and hence lack allergenic activity.

Genomics and proteonomics

The arrival of the gene chip and proteomic technologies allows the 'functional-genomic' basis of allergy to be investigated. Information on the genetics of allergic disease is valuable not only for analyzing the molecular basis of the diseases but also for the development new forms of immunotherapy. The search is on for potential candidate genes that predispose to allergy and atopy, especially those that code for and regulate IgE production and allergic inflammatory responses.⁵³ Identification and understanding of such disease-specific genes, their expression and its protein product may lead, in the future, to the development of individually tailored treatment regimens manipulating the immune system specifically for that individual.

Conclusions

The understanding of both the mechanisms and treatment of allergy has developed over the past two hundred years. Today there are exciting developments at the cellular level, which may provide future treatments. The conditions are rarely fatal but cause considerable morbidity in increasing numbers of patients. Otolaryngologists were involved with the development of this area and will continue to treat patients with rhinitis. Improved diagnosis and novel therapies will hopefully increase the number of satisfactory outcomes.

References

- 1 Blackley CH. *Experimental Researches on the Causes and Nature of Catarrhus aestivus*. 1st edn, London: Balliere, Tindall and Cox, 1873
- 2 Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911;**1**:1572-3
- 3 Freeman J. Vaccination against hay fever: report of results during the first three years. *Lancet* 1914;**1**:1178-80
- 4 Frankland AW, Augustin R. Prophylaxis of summer hay fever and asthma. A controlled trial comparing crude pollen grass extracts with the isolated main protein component. *Lancet* 1954;**1**:1055-7
- 5 Cooke RA, Van Der Veer A. Human sensitization. *J Immunol* 1916;**1**:201-37
- 6 Cooke RA, Barnard JH, Hebard S, et al. Serological evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). *J Exp Med* 1935;**62**:733-8
- 7 Hansel FK. Allergy as etiologic factor in paranasal sinus disease. *J Missouri Med Assn* 1930;**27**:275-7
- 8 Hansel FK. *Allergy of the Nose and Paranasal Sinuses*, 1st edn. St Louis: The CV Mosby Publishing Co. 1936
- 9 Rinkel HJ. The whealing response of the skin to serial dilution titration. *Ann Allergy* 1949;**7**:625-30
- 10 Van Metre TE, Adkinson NF, Lichtenstein LM, Mardiney MR, Norman PS, Valentine MD et al. A controlled study of the effectiveness of the Rinkel method of immunotherapy for ragweed pollen hay fever. *J Allergy Clin Immunol* 1980;**65**:288-97
- 11 Hirsch SR, Kalbfleisch JH, Golbert TM, Josephson BM, McConnell LH, Cohen SH et al. Rinkel injection therapy: a multicenter controlled study. *J Allergy Clin Immunol* 1981;**68**:133-55
- 12 Hirsch SR, Kalbfleisch JH, Cohen SH. Comparison of Rinkel injection therapy with standard immunotherapy. *J Allergy Clin Immunol* 1982;**70**:183-90
- 13 Van Metre TE, Adkinson NF, Amodio FJ, Kagey-Sobotka A, Lichtenstein LM, Rosenberg GL et al. A comparison of immunotherapy schedules for injection treatment of ragweed pollen hay fever. *J Allergy Clin Immunol* 1982;**69**:181-93
- 14 Van Metre TE, Adkinson NF, Amodio FJ, Lichtenstein LM, Kagey-Sobotka A, Valentine MD, et al. A comparative study of the effectiveness of the Rinkel method and in the current standard method of immunotherapy for ragweed pollen hay fever. *J Allergy Clin Immunol* 1980;**66**:500-13
- 15 Bousquet J, Lockey R, Malling HJ. WHO panel members. *J Allergy Clin Immunol* 1998;**102**:558-62
- 16 Prausnitz C, Kustner J. Studien Über Die Ueberempfindlichkeit. *Cent Bacteriol* 1921;**86**:160
- 17 Ishizaka K, Ishizaka T, Hornbrook MH. Physicochemical properties of reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. *J Immunol* 1966;**97**:75-85
- 18 Bennich HH, Ishizaka K, Johansson SG, Rowe DS, Stanworth DR, Terry WD. Immunoglobulin E: a new class of human immunoglobulin. *Immunology* 1968;**15**:323-4
- 19 Wide L, Bennich H, Johansson SG. Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* 1967;**2**:1105-7

- 20 Nalebuff DJ, Fadal RG, Ali M. RAST based immunotherapy: a new improved approach for effective allergy-specific treatment. In: Fadal RG, Nalebuff DJ, eds. *RAST in Clinical Allergy*. North Miami, Florida: USA Symposia Specialists, 1981;247-65.
- 21 Lowell FC, Franklin W. A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. *N Eng J Med* 1965;**273**:675-9.
- 22 Walker SM, Varney VA, Gaga M, Jacobson MR, Durham SR. Grass pollen immunotherapy: efficacy and safety during a 4-year follow-up study. *Allergy* 1995;**50**:405-13.
- 23 Dolz I, Martinez-Cocera C, Bartolome JM, Cimarra M. A double-blind, placebo-controlled study of immunotherapy with grass pollen extract Alutard SQ during a 3-year period with initial rush immunotherapy. *Allergy* 1996;**51**:489-500.
- 24 Bousquet J, Hejjaoui A, Skassa-Brociek W, Guerin B, Maasch HJ, Michel FB, et al. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. I. Rush immunotherapy with allergoids and standardised orchard grass-pollen extract. *J Allergy Clin Immunol* 1987;**80**:591-8.
- 25 Creticos PS, Reed CE, Norman PS, Khoury J, Adkinson N, Buncher CR, et al. Ragweed immunotherapy in adult asthma. *N Eng J Med* 1996;**344**:501-6.
- 26 Bousquet J, van Cauwenberge P, Khaltaev N eds. The WHO initiative. Allergic rhinitis and its impact on asthma (ARIA) *J Allergy Clin Immunol* 2001;**108**:S147-334.
- 27 Bousquet J, Des-Roches A, Paradis L, Dhivert H, Michel FB. Specific immunotherapy in house dust mite allergy. *Clin Rev Allergy Immunol* 1995;**13**:151-9.
- 28 Haugaard L, Dahl R, Jacobsen L. A controlled dose-response study of immunotherapy with standardised, partially purified extract of house dust-mite: clinical efficacy and side effects. *J Allergy Clin Immunol* 1993;**91**:709-22.
- 29 Alvarez-Cuesta E, Cuesta-Herranz J, Puyana-Ruiz J, Cuesta-Herranz C, Blanco-Quiros A. A monoclonal antibody-standardised cat extract immunotherapy: risk benefit effects from a double-blind placebo study. *J Allergy Clin Immunol* 1994;**93**:556-66.
- 30 Ohman JL, Findlay SR, Leitermann KM. Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of in vivo and in vitro responses. *J Allergy Clin Immunol* 1984;**74**:230-9.
- 31 Durham SR, Walker SM, Varga E, Jacobsen MR, O'Brien F, Nouri-Aria KT, et al. Long-term clinical efficacy of grass pollen immunotherapy. *N Eng J Med* 1999;**341**:468-75.
- 32 Constant SL, Bottomly K. Induction of Th1 and Th2 CD4 T cell responses: the alternative approaches. *Ann Rev Immunol* 1997;**15**:297-322.
- 33 Ryan JJ. Interleukin-4 and its receptor: essential mediators of the allergic response. *J Allergy Clin Immunol* 1997;**99**:1-5.
- 34 Mauser PJ, Pitman AM, Fernandez X, Foran SK, Adams GK, Chapman RW, et al. Effects of an antibody to interleukin-5 in a monkey model of asthma. *Am J Respir Crit Care Med* 1995;**152**:467-72.
- 35 Golden DB, Meyers DA, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Clinical relevance of the venom-specific immunoglobulin G antibody level during immunotherapy. *J Allergy Clin Immunol* 1982;**69**:489-93.
- 36 Durham SR, Till SJ. Immunological changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998;**102**:157-64.
- 37 Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR. Bee venom immunotherapy results in a decrease of IL-4 and IL-5 and increase of INF-gamma secretion in specific allergen-stimulated T-cell cultures. *J Immunol* 1995;**154**:4187-94.
- 38 Borish L. IL-10: evolving concepts. *J Allergy Clin Immunol* 1998;**101**:293-7.
- 39 Durham SR, Varney VA, Gaga M, Jacobsen MR, Varga EM, Kay AB, et al. Grass pollen immunotherapy decreases the number of mast cells in the skin. *Clin Exp Allergy* 1993;**23**:1490-6.
- 40 Ferreira F, Hirtenlehner K, Jilek A, Godrik-Cvar J, Breiteneder H, Grimm R, et al. Dissection of immunoglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v1: potential use of hypoallergenic isoforms for immunotherapy. *J Exp Med* 1996;**183**:599-609.
- 41 Ferreira F, Ebner C, Kramer B, Casari B, Briza P, Scheiner O, et al. Modulation of IgE reactivity of allergens by site-directed mutagenesis: potential use of hypoallergenic variants for immunotherapy. *FASEB J* 1998;**2**:231-42.
- 42 Takai T, Yokota T, Yasue M, Nishiyama C, Yuuki T, Okumura Y, et al. Engineering of the major house dust mite allergen Der f2 for allergen-specific immunotherapy. *Nat Biotechnol* 1997;**15**:754-8.
- 43 Briner TJ, Kuo MC, Keating KM, Rogers BL, Greenstein JL. Peripheral T-cell tolerance induced in naive mice by subcutaneous injection of peptides from the major cat allergen Fel d1. *Proc Natl Acad Sci USA* 1993;**90**:7608-12.
- 44 Higgins JA, Lamb JR, Marsh SG, Tonks S, Hayball JD, O'Hehir RE, et al. Peptide-induced non-responsiveness of HLA-DP restricted human T-cells reactive with *Dermatophagoides* spp (house dust mite). *J Allergy Clin Immunol* 1992;**90**:749-56.
- 45 Norman PS, Ohman JL, Long AA, Creticos PS, Geffter MA, Nicodemus CF, et al. Treatment of cat allergy with T-cell reactive peptides. *Am J Respir Crit Care Med* 1996;**154**:1623-8.
- 46 Maguire P, Nicodemus C, Robinson D, Aaronson D, Umetsu DT. The safety and efficacy of ALLERVAX CAT in cat allergic patients. *Clin Immunol* 1999;**93**:222-31.
- 47 Oldfield WLG, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002;**360**:47-53.
- 48 Klinzman DM, Yi AK, Beaucage SL, Conover J, Krieg AM. CpG motifs present in bacterial DNA rapidly induce lymphocytes to secrete interleukin-6, interleukin-12 and interferon-gamma. *Proc Natl Acad Sci USA* 1996;**93**:2879-83.
- 49 Raz E, Tighe H, Sato Y, Corr M, Dudler JA, Roman M, et al. Preferential induction of Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunisation. *Proc Natl Acad Sci USA* 1996;**93**:5141-5.
- 50 Casale TB, Bernstein IL, Busse WW, LeForce CF, Tinkleman DG, Stoltz RR, et al. Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. *J Allergy Clin Immunol* 1997;**158**:1438-45.
- 51 Milgrom H, Fick RB, Su JQ, Reimann JD, Bush RK, Metzga WJ, et al. Treatment of allergic asthma with monoclonal anti-IgE antibody. *N Eng J Med* 1999;**341**:1966-73.
- 52 Ganglberger E, Grunberger K, Sponer B, Radauer C, Breiteneder H, Boltz-Nitulescu G, et al. Allergen mimotopes for 3-dimensional epitope search and induction of antibodies inhibiting human IgE. *FASEB J* 2000;**14**:2177-84.
- 53 Toda M, Ono SJ. Genomics and proteomics of allergic disease. *Immunology* 2002;**106**:1-10.

Address for correspondence:

Dr D. S. Kim,
3 Foxfield,
Northfield,
Birmingham B31 1FE,
UK.

E-mail: daekim89@hotmail.com

Dr D. Kim takes responsibility of the integrity of the content of the paper.

Competing interests: None declared