

Allergy: who, why and what to do about it?

Kent T. HayGlass

Immediate hypersensitivity is the most widespread immunological disorder in humans, representing the most prevalent and rapidly increasing chronic health problem among individuals over 15 years of age¹. It affects one in four individuals in developed nations, with consequences ranging in severity from irritating to life-threatening. Furthermore, it results in huge economic losses: costing billions of dollars per annum in the USA alone². Allergen avoidance has always been the most effective therapy, but preventing exposure to common environmental substances such as house dust mite, and cat and dog allergens, is virtually impossible^{3,4}. Against this background, a recent meeting reviewed progress since the first symposium on this topic, which was held in 1992 (Ref. 5).

Availability of pure allergens

A long-standing problem in allergy research, diagnosis and treatment has been the high degree of lot-to-lot variability in commercially available (and clinically dominant) natural allergen extracts. In recent years, this problem has largely been resolved by agreement on standardized units [bioequivalent allergy units (BAU) in North America and biological units (BU) in Europe] for quantitation of allergen, rather than total protein, in any given preparation. However, this still does not overcome two major inherent liabilities of such preparations: (1) their tremendous heterogeneity; and (2) the fact that the amount of specific allergen present in most commercial extracts ranges from a few percent to 50% of the total protein present. Thus, despite advances in standardization, such extracts are still highly complex mixtures with varying amounts of major and minor allergens (and irrelevant proteins).

Considerable effort has been devoted to identifying, cloning and ex-

Many of the major allergens have been cloned and found to exhibit multiple isoforms, an observation that has important implications for the diagnosis and treatment of ongoing hypersensitivity. A recent meeting discussed this controversial area, together with the current understanding of why allergy develops and how it may be treated.*

pressing allergens of major clinical importance (reviewed in Ref. 5). The majority of the major allergens have now been cloned, and many can be expressed at moderate-to-high level as non-fusion proteins (F. Ferreira, Salzburg; W. Thomas, Perth; R. Valenta, Vienna). The imminent availability of homogeneous antigens of defined purity and biological activity is likely to have a substantial impact on diagnosis and, perhaps ultimately, on the therapy of allergic disorders. Indeed, as pointed out by R. Crameri (Davos), M. Chapman (Manchester) and G. Pauli (Strasbourg), the first recombinant allergens have already seen clinical use in Europe and the USA.

Multiple isoforms of allergens

An important finding with potentially widespread implications is the observation that many allergens exhibit multiple natural isoforms. Biochemical studies in the late 1980s suggested the existence of what is essentially allelic variation in numerous clinically important plant and fungal proteins. Now that most of the major allergens have been cloned, often in two or more laboratories at approximately the same time, there is little question that considerable microheterogeneity exists. This applies to a diverse group of proteins including Bet v 1, a major European allergen cloned from the pollen of birch trees

(Valenta; J. Larsen, Hørsholm), Cor a 1, the major allergen of hazel pollen (Ferreira), numerous grass-pollen allergens and cysteine proteinases that represent important allergens derived from house dust mites. For example, at least 12 naturally occurring isoforms of Bet v 1 have been identified (M. Breitenbach, Salzburg).

A large amount of effort is now being directed at characterizing naturally occurring and 'synthetic' isoforms created by site-directed mutagenesis (A. Bufe, Borstel; Ferreira; G. Hakkaart, Amsterdam; A. Smith, Charlottesville). These multiple variants are proving very useful in addressing two critical questions: (1) does the existence of isoforms differing in primary amino acid sequence have any relevance for the inherent allergenicity of a given protein? And (2) will they affect our approaches for diagnosis and treatment of ongoing hypersensitivity? The first question can now be evaluated using allergenic RNase mutants that have been engineered to lose their enzymatic activity and, putatively, their immunogenicity (Bufe). The second question, with its important implications for clinical management, provokes much controversy.

Diagnosis of hypersensitivity

C. Schou (Hørsholm) and S. Dreborg (Linköping) argue that the existence of many distinct isoforms of the same allergen molecule is likely to be associated with differential T- and B-cell recognition and, consequently, different allergic sensitivities. Convincing evidence was shown of the ability of antibody from different allergic subjects to bind to one, but not other, isoforms of Bet v 1 obtained from different individual birch trees growing in the same area. Similarly, Cor a 1 exists as multiple isoforms that are highly similar in their amino acid sequences and display different

*The International Symposium on 'Molecular Biology of Allergens and the Atopic Immune Response' was held in Quebec City, Canada, on 18–22 February 1995.

properties with respect to antibody binding or activation of allergen-specific T-cell clones. In some instances, isoforms of birch- and hazel-pollen allergens generated by site-directed mutagenesis (causing single amino acid substitutions) show dramatically different capacity to bind IgE from different allergic subjects (Ferreira). For many, the clear implication is that replacement of highly heterogeneous preparations of allergen from natural sources (which are likely to contain most or all allergen isoforms because they are prepared from large pools of natural material) with purified, homogeneous allergen derived from a single gene (thus, containing only a single isoform) may create as many difficulties as it solves. Identifying, cloning and expressing all potential isoforms of each allergen would be prohibitively labour intensive and expensive.

However, Thomas and D. Kraft (Vienna) strongly disagreed with this interpretation. From studies of immunodominant T-cell (J. Lamb, London; B. Wallner, Boston) or B-cell (P. Lamontagne, Quebec City; C. Suphioglu, Melbourne) epitopes of allergens using antibody or T-cell clones derived from atopic subjects, it has been inferred that most individuals respond to a remarkably limited number of epitopes on any given allergen. This is interpreted as evidence that a small panel of recombinant allergens, perhaps 40 in total (Kraft), might be sufficient to identify most allergic individuals. This prediction is based on the limited number of immunogenic proteins observed for any particular allergy and the high degree of immunological crossreactivity among different allergen isoforms. Thus, although many individuals may have their own 'customized' pattern of epitopes driving their hypersensitive state, there may be sufficient commonality within a group of, for example, birch-tree-atopic individuals to allow the vast majority of them to be diagnosed using a single isoform of the single major allergen (Bet v 1).

Treatment of hypersensitivity

The question of therapy is much more complex than that of diagnosis, and it is appreciated that the

efficacy of allergen immunotherapy is currently very low. Once (or if) a broadly effective method of allergen immunotherapy can be developed (a goal hindered at present by an uncertain understanding of the mechanisms by which immunotherapy does work when it is successful) the question of individual sensitivities will be critical. If allergen-specific immunotherapy functions by switching T helper 2 (Th2)-dominated responses into Th1-like patterns of cytokine expression, the impact of T cells that are reactive to epitopes common to the various isoforms may well dictate responses to covalently linked (isoform-unique) epitopes expressed elsewhere on the molecule (Thomas), essentially along the lines of Av Mitchison's classical carrier effect. Alternatively, if efficient therapy relies on induction of T-cell anergy [as is speculated to be a major mechanism by which peptide immunotherapy acts (Lamb, Wallner)], the reactivity of different individuals to isoform-specific determinants may require the presence of isoform-specific peptides. This could necessitate customized allergen analysis and peptide synthesis for each patient, with the impossible aim of selectively tolerizing his or her unique repertoire of allergen-reactive T cells. However, animal studies have shown that one or two immunodominant T-cell epitopes of an allergen may be sufficient to decrease allergic symptoms. Both interpretations have merit, and this question can now be addressed directly given the widespread availability of different allergen isoforms.

Who develops allergy, and why?

Despite decades of work, it is still not known why particular individuals develop allergy. D. Marsh (Baltimore) reviewed the difficulties encountered when using segregation analysis to determine the major genes that are responsible for induction of the allergic state. The most prominent are the many confounding variables (including time, nature and intensity of allergen exposure, age and sex) that complicate analysis of phenomena controlled by multiple genes. Much effort continues to be concentrated on identification of candidate genes by looking for polymorphisms and

their association with the atopic state (W. Cookson, Oxford).

At a separate level of control, substantial progress has been made in identifying the immunological mechanisms underlying the initial induction and control of interleukin 4 (IL-4) (K. Murphy, St Louis), IL-12 (M. Kapsenberg, Amsterdam) and interferon γ (IFN- γ) (K. HayGlass, Winnipeg) responses. It is well recognized that the cytokine environment in which naive T cells are initially activated is pivotal in determining whether the immune response that evolves is clinical tolerance or hypersensitivity. Identification of these elements is likely to prove a major area of interest over the next few years. Moreover, despite the unquestioned importance of differential activation of Th1-versus Th2-like patterns of cytokine expression among CD4⁺ T cells (S. Romagnani, Florence; Q. Hamid, Montreal; J. de Vries, Palo Alto) in the induction and maintenance of allergic responses, other cell types, in particular mast cells (D. Befus, London) and eosinophils (R. Moqbel, Edmonton), are likely to play an equally critical role. Whether in ongoing allergic responses or perhaps as initial producers of IL-4 following interaction with proteolytic enzymatic allergens, antigen-nonspecific regulation of cytokine synthesis is an area requiring more investigation. Sources of Th2-like cytokines may prove to be a major barrier to attempts at redirecting antigen-specific (i.e. T-cell mediated) cytokine synthesis in allergic individuals. In this regard, the data of A. Schon (Winnipeg) demonstrating inactivation of mast cells *in vitro* and *in vivo* with monomethoxy polyethylene glycol (mPEG)-conjugated allergens may be promising.

Novel therapeutic approaches currently under evaluation

Some nine decades after the first attempts at allergic 'desensitization', a broad range of therapeutic strategies is now in development. Wallner and P. Norman (Baltimore) presented data derived from basic research and clinical trials, respectively, on the efficacy of antigen-specific peptide immunotherapy for cat allergy, the most common inhalant

allergen worldwide. Preliminary data suggest that treatment with high doses of Feld1-derived peptides results in decreased sensitivity, since treated subjects could withstand longer periods of provocation with high levels of environmental cat allergens than control subjects (although there were no detectable immunological changes in the parameters examined). These studies are entering Phase III trials, but caution needs to be exercised in interpreting the effects prematurely.

As an alternative approach, B. Stadler (Bern) and C. Heusser (Basel) argue for the potential of anti-IgE immunotherapy, based on the rationale that anti-IgE antibodies are constitutively present in most subjects and have been suggested to act as a natural 'emergency brake' on spontaneous IgE synthesis. Clinical trials are currently being performed using humanized anti-IgE antibodies that are capable of preventing newly synthesized IgE from binding to mast cells or basophils, without triggering widespread activation by crosslinking IgE already bound by the high-affinity IgE receptor (P. Jardieu, San Francisco; F. Davis, Houston). Preliminary results look exciting, but ongoing therapy is likely to be required.

In other approaches, de Vries presented data suggesting the potential therapeutic use of mutant recombinant (r)IL-4, and J. Hebert (Quebec City) argued for further investigation of anti-idiotypic-based IgE regulation. There is little doubt that pharmacological approaches are currently in favour for patient management, but the immunological strategy that is most likely to succeed in dealing with the underlying disease state remains to be determined.

Future work

The greatest handicap in developing immunotherapeutic approaches is that we still have an uncertain understanding of: (1) the specific factors that lead to initial induction of immediate hypersensitivity responses *in vivo*; and (2) what is required to reorient an ongoing immune response. This same challenge hinders development of immunization protocols and therapies for various infectious diseases [such as leprosy, several parasitic diseases

and perhaps AIDS (Refs 6–9)] where skewing of the T-cell response in an appropriate direction is recognized as a primary cause of pathological changes and, ultimately, a worsened clinical outcome. Given the speed at which new hypotheses are being formulated (and often discarded), it is very likely that our efforts to steer *de novo* and ongoing immune responses *in vivo* can be more precisely targeted by the time of the next meeting in 1998.

Recognition that it is much easier to tolerize naive T cells, or guide them towards preferred (protective) patterns of cytokine synthesis, in individuals that are naive for a given antigen has stimulated recent discussion of prophylactic hyposensitization of young children. However, several concerns arise. The number of potential allergens required is yet to be determined but could be very great. Treatment schedules would have to be constructed to act during a childhood window that seems to correspond to increased susceptibility of hypersensitivity induction. At the same time, one needs to avoid high-risk periods such as that near DPT (diphtheria, pertussis, tetanus) immunization (E. Gelfand, Denver), a stage at which the potential of pertussis to act as a Th2-promoting adjuvant for allergens would need to be guarded against. Furthermore, because of counter-intuitive findings that prevention is not necessarily more efficient than treatment¹⁰, the willingness of parents and health-care administrators to participate in such a scheme remains to be seen. However, for children of two asthmatic parents, the probability of asthma is 70–80% (Gelfand) and prophylaxis may be justified in this high-risk group. Furthermore, if genetic and epidemiological studies could identify additional common target groups at elevated risk for hypersensitivity, the feasibility of this strategy would be substantially increased.

In closing the meeting, Kraft highlighted the link between the decline of childhood worm infections, which used to be common even in developed nations, and the continuously increasing frequency of hypersensitivity diseases. Together with the striking inverse correlation between

total serum IgE levels and atopic disease in parasitized versus 'healthy' populations (J. Faux, Oxford), the point was clearly made that an improved standard of living carries with it some liabilities.

I thank D. Kraft, J. Lamb, A. Sehon and W. Thomas for critical review of the manuscript. My apologies to my colleagues whose contributions could not be discussed here because of space limitations.

Kent HayGlass is at the Dept of Immunology, 730 William Ave, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

References

- 1 Health Status of Canadians: Report of the 1991 General Social Survey (1994), pp. 175–193, *Statistics Canada*
- 2 Holt, P.G. (1994) *Immunol. Today* 15, 484–489
- 3 Wood, R.A., Mudd, K.E. and Eggleston, P.A. (1992) *J. Allergy Clin. Immunol.* 89, 126–130
- 4 Munir, A.K.M., Einarsson, R., Schou, C. and Dreborg, S.K.G. (1993) *J. Allergy Clin. Immunol.* 91, 1067–1074
- 5 Kraft, D. and Sehon, A. (eds) (1993) *Molecular Biology and Immunology of Allergens*, CRC Press
- 6 Pirmez, C., Yamamura, M., Uyemura, K., Paes-Oliveira, M., Conceição-Silva, F. and Modlin, R.L. (1993) *J. Clin. Invest.* 91, 1390–1395
- 7 Salk, J., Bretscher, P.A., Salk, P.L., Clerici, M. and Shearer, G.M. (1993) *Science* 260, 1270–1272
- 8 Modlin, R.L. (1994) *J. Invest. Dermatol.* 102, 828–832
- 9 Romagnani, S. (1994) *Annu. Rev. Immunol.* 12, 227–257
- 10 Leutwyler, K. (1995) *Sci. Am.* 272, 124–129

Next month in *IT*

- Costimulation through Fas and FasL
- BSAP and B-cell development
- Immunosuppressive retroviral peptides
- TCR usage in human tumours