

Rudolf Valenta
Dietrich Kraft

Recombinant allergen molecules: tools to study effector cell activation

Authors' address

Rudolf Valenta, Dietrich Kraft,
Department of Pathophysiology, Vienna
General Hospital, University of Vienna,
Vienna, Austria.

Correspondence to:

Rudolf Valenta
"Molecular Immunopathology Group"
Department of Pathophysiology
University of Vienna Medical School
Vienna General Hospital
Waehringer Guertel 18–20
A-1090 Vienna
Austria
Tel: 43 1 40400 5108
Fax: 43 1 40400 5130
e-mail: Rudolf.Valenta@akh-wien.ac.at

Acknowledgements

The authors would like to thank Dr. Peter Valent, Department of Hematology, Vienna General Hospital, Austria, and Dr. Steven C. Almo, Department of Biochemistry, Albert Einstein College of Medicine, New York, USA, for fruitful collaboration regarding effector cell activation and structural biology, respectively. The skilful editorial assistance of Irmgard Lubelik is acknowledged. This work was supported by grant Y078 GEN of the Austrian Science Fund and by the ICP Program of the Austrian Federal Ministry for Education, Science and Culture.

Summary: The IgE antibody-mediated activation of allergic effector cells is the key pathomechanism underlying the immediate symptoms of Type I allergy, a genetically determined hypersensitivity disease affecting 25% of the population. In recent years important environmental allergens and their epitopes have become available as structurally defined recombinant molecules. In addition, corresponding human monoclonal IgE and IgG antibodies have been isolated. This review summarizes data obtained regarding the three-dimensional structure of allergens, their IgE epitopes and the recognition of allergens by IgE and IgG antibodies. In particular, we discuss results of recent *in vitro* and *in vivo* studies with defined allergen molecules, their epitopes and the corresponding antibodies which support the hypothesis that the density and geometrical arrangement of IgE epitopes on a particular allergen molecule may profoundly affect effector cell activation. If the structural requirements for effector cell activation can be delineated, it may be envisaged that, based on this knowledge, allergens can be converted into hypoallergenic immunogens by reorientation of IgE epitopes. Such allergen derivatives may be used for allergen-specific immunotherapy with reduced risk of inducing anaphylactic side effects.

Introduction

The immediate symptoms (e.g. allergic rhinoconjunctivitis, allergic bronchial asthma, anaphylactic shock) of Type I allergy, an IgE-mediated hypersensitivity disease, result from the cross-linking of effector cell-bound IgE antibodies by otherwise harmless environmental antigens (i.e. allergens). The cross-linking of effector cell-bound IgE antibodies is achieved through binding of the paratopes of at least two IgE antibodies to two epitopes on an allergen molecule (1). In the past it was difficult, if not impossible, to obtain homogeneous preparations of defined allergen molecules and the corresponding IgE antibodies. Therefore, experimental studies on effector cell activation were performed using chemically cross-linked IgE complexes, anti-IgE antibodies, natural allergen extracts or allergen preparations consisting of a variety of allergens and isoallergens, respectively, or artificial aller-

Immunological Reviews 2001
Vol. 179: 119–127
Printed in Denmark. All rights reserved

Copyright © Munksgaard 2001
Immunological Reviews
ISSN 0105-2896

gens with little relevance for human disease (2–6). Nevertheless, many of the mechanisms involved in IgE-mediated effector cell activation have been revealed. Amazingly, fewer than 100 antigen molecules involving less than 15% of effector cell-bound IgE antibodies were found to induce optimal histamine release from human basophils (6, 7). Furthermore, it has been demonstrated that dimerization of IgE antibodies can serve as the principal signal for effector cell activation and release of biologically active mediators (1). However, when IgE-mediated histamine release from human basophils induced with anti-human IgE antibodies was compared with that induced with natural allergen preparations, significant differences were noted (5). It was found that human basophils were activated by much lower concentrations of antigen than anti-IgE antibodies and that antigen triggered histamine release over a wide range of concentrations, whereas anti-IgE antibody-induced release occurred over a more limited concentration range. Furthermore, release by antigen occurred with a shorter lag period and a more rapid rate than that observed with anti-IgE. These differences in initiation of histamine release were attributed to the fact that antigens represent multivalent ligands, whereas anti-IgE is functionally bivalent (5).

During the last decade the molecular nature of many important environmental allergens has been revealed with the implementation of molecular biology techniques for the characterization of allergens (8). Several laboratories have started to isolate cDNAs coding for important environmental allergens and to use them for the production of defined recombinant allergen molecules which are immunologically equivalent to their natural counterparts (9). Primary allergen sequences provided information regarding the molecular nature of allergens, allowed the mapping of allergen epitopes, and facilitated the determination of three-dimensional allergen structures (reviewed in 10). The number of allergen structures becoming available continues to increase, and we are beginning to learn about the number and geometry of IgE epitopes on important environmental allergens.

Three-dimensional structure analysis and IgE epitope mapping of allergens

Recombinant DNA technology has advanced both the structural analysis of allergens and the characterization of IgE epitopes (10). Homogeneous recombinant allergen preparations could be obtained by expression of the allergen-encoding DNAs in bacteria or in eukaryotic cells. The purified recombinant allergens were analyzed by X-ray crystallography or,

when produced as metabolically labeled proteins, by nuclear magnetic resonance (NMR) spectroscopy. NMR analysis represents a straightforward technology particularly suitable for the analysis of allergens, because many of them they represent small proteins with a molecular weight in the NMR range (<30 kDa).

Table 1 summarizes allergens whose three-dimensional structure has been determined. All of these allergens represent proteins or glycoproteins. IgE antibodies of allergic patients are mainly directed against the protein backbone of these allergens. They may recognize conformational structures assembled by discontinuous peptides or amino acids and/or continuous peptide epitopes. In contrast to T-cell epitopes which are small peptides resulting from the proteolytic processing of allergens, IgE epitopes represent surface-exposed allergen structures. So far, the three-dimensional structures of several important animal-derived, plant- and dust-mite allergens have been revealed (Table 1). The biological functions of these allergens are diverse or unknown, and there seems to be no particular functional feature which would predispose a protein to behave as an allergen. The mammalian allergens with known or assumed biological function comprise parvalbumin, a calcium-binding muscle protein (11, 12), albumin, an abundant serum protein (13, 14) and the major allergens from cattle and horse dander, Bos d 2 and Equ c 1, respectively (15, 16). The latter belong to a family of lipid-binding proteins, the lipocalins. Bet v 1, the major birch pollen allergen, exhibits sequence homology with pathogenesis-related (PR) plant proteins, which are activated upon stress, exposure to certain hormones or infections in plants (17, 18). Plant profilins, as represented by the birch pollen allergen, Bet v 2, belong to probably the most cross-reactive allergens known to date (19–22). They represent actin-binding proteins which also interact with phospholipids.

The three-dimensional structures of the allergens summarized in Table 1 are also very diverse. Several allergens (e.g. parvalbumin, Bos d 2, Equ c 1, Amb t 5, Bet v 1, Bet v 2) contain a mixed α -helical-, β -sheet fold, whereas others consist mainly of α -helices (e.g. albumin, Phl p 5) or even mimic an immunoglobulin-line fold of exclusive β -sheet structure (e.g. Phl p 2, Der p 2, Der f 2) (11, 14–16, 18, 21–27).

Thus there appears to be no apparent common structural theme among different allergens which is associated with their intrinsic property to elicit an allergic immune response.

However, one interesting observation emerging from the structural analysis of allergens is that they can exhibit structural similarities with each other or with human proteins which in part could not be predicted according to their pri-

Table 1. Allergens with available three-dimensional structure. Allergens (designation), their sources and biological function are listed. Furthermore, prevalence of IgE recognition, fold, type of IgE epitopes and references are displayed

Allergen	Source	Biological function	Prevalence	Fold	IgE epitopes	Reference
Parvalbumin	carp muscle	Ca ²⁺ -binding protein	>90% of fish allergics	α/β	discontinuous	11, 12
Albumin	mammals	serum protein	30% of animal allergics	α	continuous and discontinuous	13, 14
Bos d 2	cattle	lipocalin	>50% of cattle allergics	α/β	discontinuous	15
Equ c 1	horse	lipocalin	>50% of horse allergics	α/β	discontinuous	16
Amb t 5	ragweed pollen	unknown	>50% of ragweed allergics	α/β	n.k.	23
Bet v 1	birch pollen	homologous to PR proteins	>95% of birch pollen allergics	α/β	discontinuous	17, 18
Bet v 2 (profilin)	birch pollen	actin-, PIP2-binding	10% of plant allergics	α/β	continuous and discontinuous	21
Arabidopsis profilin	Arabidopsis	actin-, PIP2-binding	10% of plant allergics	α/β	continuous and discontinuous	22
Phl p 5	timothy grass pollen	RNase?	80% of grass pollen allergics	α	continuous and discontinuous	24
Phl p 2	timothy grass pollen	unknown	70% of grass pollen allergics	β	continuous and discontinuous	25
Der p 2	mite	unknown	>80% of mite allergics	β	discontinuous	26
Der f 2	mite	unknown	>80% of mite allergics	β	discontinuous	27

α : α -helix; β : β -sheet; n.k.: not known.

mary sequence (e.g. allergens with immunoglobulin-like folds: Phl p 2, Der p 2, Der f 2) (25–27).

Regarding the type of IgE epitopes, we have basically used the terms “continuous” and “discontinuous”. The term “continuous epitope” defines an allergen portion (e.g. recombinant allergen fragment, synthetic allergen-derived peptide) which consists of a row of consecutive amino acids, whereas the term “discontinuous epitope” defines IgE epitopes which are assembled by amino acids or peptides from different portions of the allergen that are brought into proximity through folding of the allergen molecule. The fact that small allergen fragments produced as recombinant or synthetic peptides bind IgE antibodies indicates the presence of continuous IgE epitopes on an allergen molecule (28–31). If it is impossible to identify IgE-reactive allergen fragments, it may equally well be the case that the allergen contains discontinuous IgE epitopes or that IgE antibodies require properly folded epitopes for binding (32–34). In the first case, fragmentation has caused dissociation of the epitope subunits, whereas in the second case, loss of the molecules or epitopes fold/conformation is the reason for lack of IgE binding.

It is thus obvious that the precise analysis of IgE epitopes of allergens represents a difficult task, both because IgE antibodies represent the least abundant class of immunoglobulins and because of the technical limitations of the experimental analysis of discontinuous and conformational epitopes. In the

case of two allergens, profilin, the highly cross-reactive plant allergen and the major timothy grass pollen allergen, Phl p 5, it has been possible to obtain independent information about the three-dimensional structure of the allergen and the position and nature of IgE epitopes. Both, for profilin and for Phl p 5, several recombinant allergen fragments have been identified which react with patients' IgE antibodies. The results of the IgE epitope mapping experiments identified at least three different IgE epitope-containing areas on each of the two allergens (21, 24, 31, 35).

Profilin is an actin-binding protein, which interacts also with poly-L-proline and phospholipids (Fig. 1). It consists of a mixed α -helical and β -sheet conformation. Interestingly, the IgE epitope-containing areas overlap with those portions of profilin, which are involved in binding to actin, the most important natural partner protein (Fig. 1) (21). This finding provides two kinds of information: first, it strongly suggests that the ligand-free profilin molecule must have sensitized patients, leading to IgE antibodies against profilin; and second, it indicates that surface-exposed areas of the allergen which have affinity for natural ligands are recognized by the variable regions of IgE antibodies (21).

Fig. 2 shows that despite their small size (5–60 kDa), allergens such as profilin can simultaneously bind multiple IgE antibodies of different specificity. Birch-profilin, a 14-kDa molecule, can easily accommodate binding of at least three differ-



Fig. 1. Model of the birch pollen profilin structure showing the IgE- and ligand-binding sites. The profilin backbone is coloured in white. Experimentally mapped IgE epitopes are displayed in green. The actin-binding surface comprises the C-terminal helix and the three bottom strands of the β -sheet. A yellow poly-L-proline molecule has been inserted to indicate the poly-L-proline binding site.

ent antibody molecules (Fig. 2). The fact that within the IgE epitope-containing region of profilin several different IgE-reactive portions were identified suggests that even more than three different IgE antibodies can simultaneously bind to this allergen.

Similar results were obtained for Phl p 5, which in contrast to profilin, presents an all α -helical protein (24, 35): IgE-reactive Phl p 5 fragments represented surface-exposed areas. When we analyzed an IgE-reactive recombinant Phl p 5 fragment comprising approximately one third of the 30-kDa allergen, we found that this fragment must contain binding sites for several different IgE antibodies. Polyclonal IgG antibodies but not a monoclonal IgE-antibody obtained from a Phl p 5-allergic patient inhibited the binding of polyclonal Phl p 5-specific IgE antibodies to this allergen domain (35, 36). Results from the structural analysis of allergens and their epitopes thus show that important environmental allergens contain multiple IgE epitopes which cluster at exposed surface areas of the molecules and allow the simultaneous binding of several (>2) IgE antibodies with different specificity. Moreover, there is evidence that these surface-exposed epitope-containing areas, although small in size, may contain several densely packed IgE epitopes (35).

Evidence for the reversible plasticity of allergen molecules

Experimental results from the analysis of the three-dimensional structure and IgE epitopes of important environmental allergens largely confirm our expectations and current hypotheses regarding the IgE recognition of allergens, although it is quite surprising that small molecules such as profilin or their fragments can simultaneously bind several IgE antibodies with different epitope specificity.

We would now like to discuss another, less expected property of allergen molecules which we summarize under the term “reversible plasticity of allergen molecules”. In this context, “reversible plasticity” means that an allergen can reversibly assume different conformations which expose IgE epitopes to a varying degree. In other words, it is possible that the same allergen molecule can change from a conformation exposing many IgE epitopes to one exposing only a few IgE epitopes. Experimental support for the reversible plasticity of allergen molecules comes from two directions.

The first observation suggesting reversible plasticity of allergens was made when the IgE-binding properties of calcium-binding pollen allergens were analyzed.

Bet v 3, a calcium-binding birch pollen allergen, which

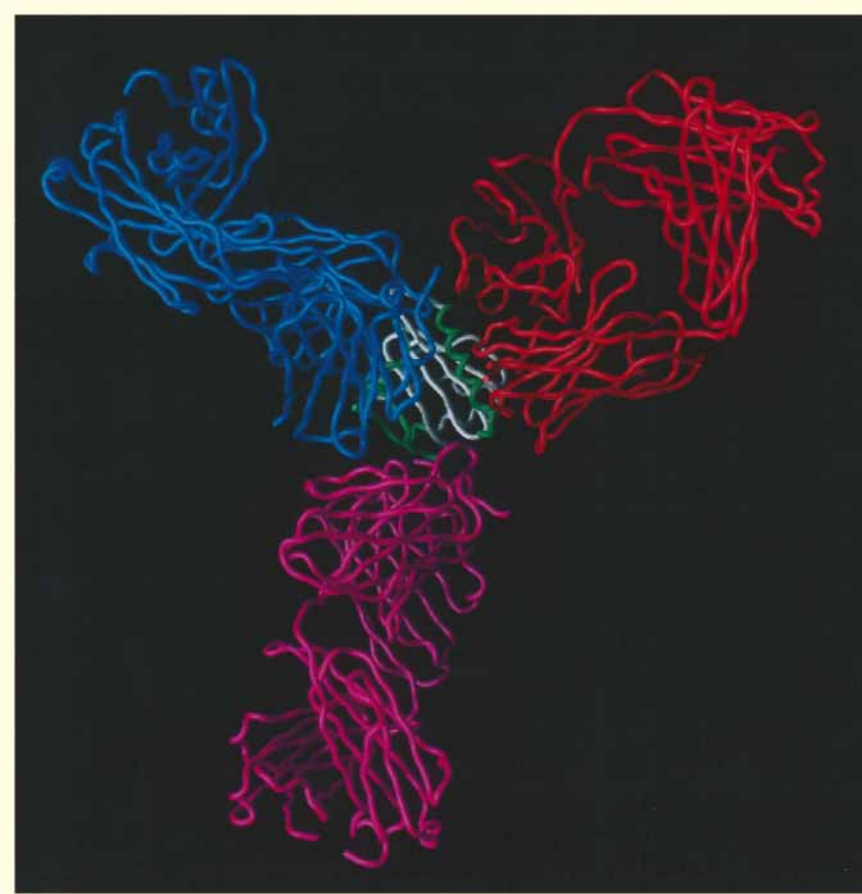


Fig. 2. Model of the simultaneous binding of three antibodies to birch pollen profilin. The profilin backbone is in white with the IgE epitopes in green. Fab fragments (red, blue, magenta) are modeled to contact the three major epitopic regions with minimal steric hindrance between the antibody molecules. Despite the small size of profilin, the distribution of epitopes should allow for the simultaneous binding of multiple IgE antibodies.

contains three calcium-binding sites (EF-hands) was found to exhibit a strongly reduced IgE-binding capacity when calcium was depleted from the protein by EGTA treatment (37, 38). This behavior was reversible because reconstitution of the molecule with calcium restored its IgE-binding capacity.

Calcium-mediated modulation of the IgE-binding capacity has meanwhile been demonstrated for several calcium-binding allergens from plants and fish (reviewed in: 39, 40). The most likely explanation for this phenomenon is that calcium-binding proteins can occur in calcium-bound (open) and calcium-free (apo-closed) forms, which assume different conformations. Amino acids being exposed on the open form will be buried in the closed apo-form, and thus can be recognized by IgE antibodies depending on the presence of protein-bound calcium ions (41–44).

The observation that the apo-forms of calcium-binding allergens mostly showed reduced IgE-binding capacity suggested production of mutated versions of these allergens which cannot bind calcium and thus have reduced IgE-

binding capacity for safe immunotherapy (40, 42). Such mutants do indeed show reduced IgE-binding capacity and exhibit reduced allergenic activity, as demonstrated by their reduced ability to induce histamine release from basophils of sensitized patients (Twardosz & Valenta, unpublished data).

The second line of evidence for reversible plasticity of allergens comes from the observation that binding of IgG antibodies to the major birch pollen allergen, Bet v 1, enhanced IgE recognition of this allergen (45–48). Definitive proof for the induction of a conformational change of the Bet v 1 structure through the attachment of IgG antibodies is still lacking. Nevertheless, the fact that human Bet v 1-specific IgG Fabs enhanced IgE recognition and Bet v 1-induced skin reactions strongly suggests a conformation-dependent exposure of IgE epitopes as the possible mechanism behind this phenomenon (48). Should further structural analysis of calcium-binding allergens and Bet v 1-IgG complexes support our hypothesis of reversible allergen

plasticity, we must consider certain allergens as “dynamic” molecules. These allergens (Bet v 1, calcium-binding allergens) can occur in different conformations, which can expose IgE epitopes to a varying degree. These conformations can be subject to reversible changes and may have different biological activity (e.g. activation of effector cells).

The discrepancy between serum IgE reactivity and biological activity revealed with recombinant allergen molecules

It is well established that rises in IgE antibody titers induced by allergen contact are associated with increased sensitivities to the given allergen as measured in skin reaction (49). On the other hand, it has been reported that specific serum IgE levels are not correlated with the magnitude of skin sensitivity (50). The question of whether measurement of specific serum IgE antibody levels is sufficient to determine the sensitivity of allergic patients is therefore a controversial issue (51). Investigations regarding the association of allergen-specific serum IgE antibody titers and sensitivity have been hampered by the lack of pure allergens. Recent studies performed with purified recombinant allergen molecules provide strong evidence for a discrepancy between serum IgE reactivity and biological activity at a molecular level. In this context it was noted that allergen-specific serum IgE antibody levels to recombinant birch and grass pollen allergens were poorly correlated with skin sensitivity (50). A subsequent study further investigating this observation showed that skin and nasal sensitivity, but not serum IgE levels to allergen molecules showed an excellent correlation (52). The discrepancy observed between biological activity and serum IgE levels could not be attributed to the presence of specific IgG antibodies interfering with the IgE recognition (52). Basophil histamine release experiments performed with purified recombinant allergen molecules and their fragments confirmed the discrepancy between serum IgE reactivity and biological activity (35). Allergens with comparable IgE-binding capacity induced strongly varying effector cell responses as measured by histamine release experiments (35). Given the reasonable assumption that the profile of specific IgE antibodies in serum closely resembles that of basophil (effector cell)-bound IgE, it must be concluded that parameters other than IgE antibody levels can influence effector cell activation. Further support for this assumption comes from the observation that certain IgE-reactive allergen molecules are weak inducers of effector cell activation (53). Moreover, a recombinant derivative of the major

birch pollen allergen, Bet v 1, was found to exhibit a more than 100-fold reduced ability to induce histamine release from basophils of Bet v 1-sensitized patients, and likewise it induced only weak skin reactions. This hypoallergenic Bet v 1-derivative was obtained by engineering a stable recombinant Bet v 1 trimer, which, despite its strongly reduced allergenic activity, reacted with Bet v 1-specific IgE antibodies (Vrtala & Valenta, unpublished data) (54, 55).

The observations made with purified recombinant allergen molecules strongly suggest that the amount of specific serum, and thus presumably that of effector cell-bound IgE antibodies, cannot predict the allergenic potential of a given allergen molecule. This hypothesis is in good agreement with previous data obtained by anti-IgE-induced effector cell activation showing that the number of IgE molecules on an effector cell will not necessarily dictate the magnitude of degranulation (7). The capacity of effector cells to respond differently to anti-IgE-induced stimulation was at that time described by the term “releasability”. Different releasability can, however, not explain different sensitivity to two different allergen molecules with comparable IgE-binding capacity in the same individual, and thus in the same preparations of effector cells.

Possible answers to the phenomenon of dissociated IgE-binding capacity and biological activity of allergen molecules

The following two observations highlight the dissociation of IgE-binding capacity and biological activity of allergen molecules:

1) Basophils from grass pollen allergic patients released histamine much more strongly in response to one recombinant grass pollen allergen fragment (rPhl p 5) than to another recombinant grass pollen allergen (rPhl p 6), although both molecules exhibited comparable IgE-binding capacity and had similar secondary structure and fold (35).

2) Basophils from birch pollen-allergic patients released more than 1000-fold less histamine when exposed to a recombinant stable trimer of the major birch pollen allergen, Bet v 1 (Bet v 1 trimer) than to the recombinant Bet v 1 monomer, although both molecules contained similar IgE epitopes and assumed comparable fold (54, 55, Vrtala and Valenta, unpublished data).

In both cases it seems unlikely that effector cell responses were influenced by antibodies of isotypes other than IgE, because sera of the tested patients did not contain detectable allergen-specific IgG- or IgA-antibodies. Furthermore, basophil histamine release experiments were performed after re-

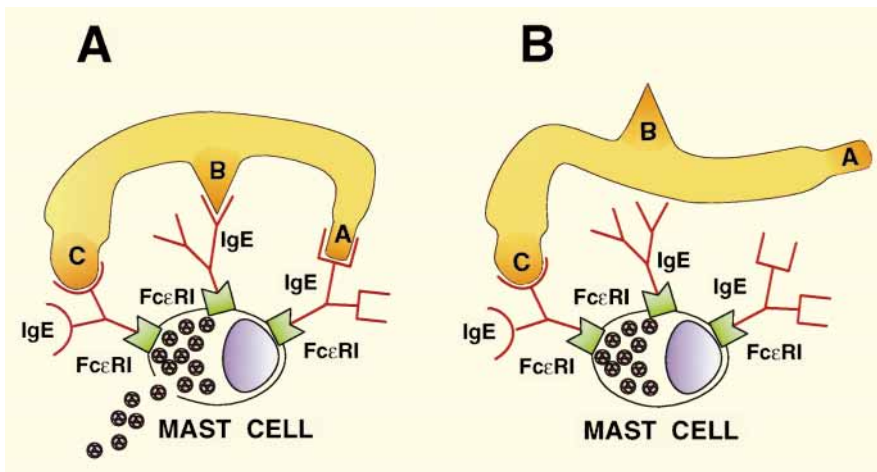


Fig. 3. The possible influence of IgE epitope geometry and density on effector cell activation. Different orientation of the same IgE epitopes (A, B, C) on an allergen may either optimally cross-link effector cell (mast cell)-bound IgE antibodies (left part; A) or fail to induce IgE-mediated cross-linking of FcεRI (right part; B). A molecule containing the same IgE-binding sites may thus either strongly induce histamine-release (A: left) or behave like a hapten (B: right).

removal of IgG-containing serum. The possibility that serum contains allergen-specific IgE antibodies with varying ability to bind to and activate effector cells which are equally well measured by serology remains to be investigated. Nevertheless, our observations are in agreement with earlier observations that: (i) allergens represented more potent activators of effector cell activation than dimerizing anti-IgE antibodies (5); and (ii) different anti-FcεRI antibodies exhibited a strongly varying capacity to induce histamine release (56).

On the basis of these observations, we propose that the density of different IgE epitopes and their orientation on a given allergen molecule will determine its capacity to induce effector cell activation. According to this hypothesis, allergens containing a large number of different IgE epitopes arranged densely and in an optimal conformation will represent potent inducers of effector cell activation (Fig. 3A). Allergens containing fewer IgE epitopes or a comparable number of different IgE epitopes with a less favorable geometric arrangement (Fig. 3B) will be poor activators of mediator release. Our hypothesis that conformational constraints determine the efficacy of effector cell activation is in agreement with the observation that signaling through cytokine receptors depends critically on receptor orientation (57). In this context it is noteworthy that signal transduction through the high-affinity receptor for IgE on basophils, and possibly through related receptors on other hematopoietic cells, appears to be enhanced by association with rafts (58, 59). It may well be that IgE epitopes on allergens can cluster FcεRI via the corresponding IgE antibodies in such membrane microdomains, with varying efficacy depending on geometrical constraints. At present, definitive experimental proof is lacking for the proposed explanation, but observations made

with defined recombinant allergen molecules, and much earlier with co-valently linked IgE oligomers, anti-IgE-antibodies and anti-FcεRI antibodies, strongly suggest a role of epitope density and geometry in effector cell activation.

Development of hypoallergenic allergen derivatives for immunotherapy by reorientation of IgE epitopes

If studies performed with structurally defined IgE epitope constructs and monoclonal IgE antibodies with defined epitope specificity should reveal that certain assemblies of IgE epitopes are potent effector cell activators, whereas others bind IgE antibodies but are poor inducers of mediator release, the development of new therapeutics may be envisaged. A major problem of allergen-specific immunotherapy is that the administration of biologically active allergen molecules can induce anaphylactic side effects. The modification of therapeutic allergen preparation by chemistry, molecular biology and peptide synthesis techniques has therefore been used to reduce the allergenic potential of therapeutic allergen preparations (reviewed in 60). However, when the allergenic potential is reduced by destroying or removing IgE epitopes, the immunogenicity of the molecules decreases. Allergen modifications with reduced IgE-binding capacity may therefore be less potent in inducing protective IgG antibody responses to IgE epitopes. In this context it would be useful if strategies could be developed which would allow the conversion of highly allergenic molecules into hypoallergenic derivatives through reorientation of IgE epitopes. Such derivatives would exhibit a reduced risk of inducing anaphylactic side effects and preserve their immunogenic features.

References

- Segal DM, Taurog JD, Metzger H. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc Natl Acad Sci USA* 1980;**74**:2993–2997.
- Fewtrell C, Metzger H. Larger oligomers of IgE are more effective than dimers in stimulating rat basophilic leukemia cells. *J Immunol* 1980;**125**:701–710.
- Kagey-Sobotka A, Dembo M, Goldstein B, Metzger H, Lichtenstein LM. Qualitative characteristics of histamine release from human basophils by covalently cross-linked IgE. *J Immunol* 1981;**127**:2285–2291.
- Dembo M, Kagey-Sobotka A, Lichtenstein L, Goldstein B. Kinetic analysis of histamine release due to covalently linked IgE dimers. *Mol Immunol* 1982;**19**:421–434.
- Marone G, Kagey-Sobotka A, Lichtenstein LM. IgE-mediated histamine release from human basophils: differences between antigen E- and anti-IgE-induced secretion. *Int Arch Allergy Appl Immunol* 1981;**65**:339–348.
- MacGlashan D Jr, Lichtenstein LM. Studies of antigen binding on human basophils. I. Antigen binding and functional consequences. *J Immunol* 1983;**130**:2330–2336.
- Conroy MC, Adkinson F Jr, Lichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977;**118**:1317–1321.
- Valenta R, Kraft D. Recombinant allergens for diagnosis and therapy of allergic diseases. *Curr Opin Immunol* 1995;**7**:751–756.
- Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;**29**:896–904.
- Valenta R, et al. The immunoglobulin E-allergen interaction: a possible target for therapy of Type I allergic diseases. *Int Arch Allergy Immunol* 1998;**116**:167–176.
- Kumar VD, Lee L, Edwards BF. Refined crystal structure of calcium-liganded carp parvalbumin 4.25 at 1.5-Å resolution. *Biochemistry* 1990;**29**:1404–1412.
- Bugajska-Schretter A, et al. Purification, biochemical, and immunological characterization of a major food allergen: different immunoglobulin E recognition of apo- and calcium-bound forms of carp parvalbumin. *Gut* 2000;**46**:661–669.
- Pandjaitan B, Swoboda I, Brandejsky-Pichler F, Rumpold H, Valenta R, Spitzauer S. *Escherichia coli* expression of recombinant dog albumin, a cross-reactive animal allergen. *J Allergy Clin Immunol* 2000;**105**:279–285.
- Curry S, Mandelkow H, Brick P, Franks N. Crystal structure of human serum albumin complexed with fatty acid reveals and asymmetric distribution of binding sites. *Nat Struct Biol* 1998;**5**:827–835.
- Rouvinen J, et al. Probing the molecular basis of allergy. Three dimensional structure of the bovine lipocalin allergen Bos d 2. *J Biol Chem* 1999;**274**:2337–2343.
- Lascombe MB, et al. Crystal structure of the allergen Equ c 1. A dimeric lipocalin with restricted IgE-reactive epitopes. *J Biol Chem* 2000;**275**:21572–21577.
- Breiteneder H, et al. The gene coding for the major birch pollen allergen, Bet v 1, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989;**8**:1935–1938.
- Gajhede M, et al. X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. *Nat Struct Biol* 1996;**3**:1040–1045.
- Valenta R, et al. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991;**253**:557–560.
- Valenta R, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;**175**:377–385.
- Fedorov AA, Ball T, Mahoney NM, Valenta R, Almo SC. The molecular basis for allergen cross-reactivity: crystal structure and IgE-epitope mapping of birch pollen profilin. *Structure* 1997;**5**:33–45.
- Thorn KS, et al. The crystal structure of a major allergen from plants. *Structure* 1997;**5**:19–32.
- Metzler WJ, Valentine K, Roebber M, Friedrichs MS, Marsh DG, Mueller L. Determination of the three-dimensional solution structure of ragweed allergen Amb t V by nuclear magnetic resonance spectroscopy. *Biochemistry* 1992;**31**:5117–5127.
- Bufe A, et al. Crystallization and preliminary diffraction data of a major pollen allergen: crystal growth separates a low molecular weight form with elevated biological activity. *J Biol Chem* 1996;**271**:27193–28196.
- De Marino S, et al. An immunoglobulin-like fold in a major plant allergen: the solution structure of Phl p 2 from timothy grass pollen. *Structure* 1999;**7**:943–952.
- Mueller GA, Benjamin DC, Rule GS. Tertiary structure of the major house dust mite allergen Der p 2: sequential and structural homologies. *Biochemistry* 1998;**37**:12707–12714.
- Ichikawa S, et al. Solution structure of Der f 2, the major mite allergen for atopic diseases. *J Biol Chem* 1998;**273**:356–360.
- Greene WK, Cyster JG, Chua KY, O'Brien RM, Thomas WR. IgE and IgG binding of peptides expressed from fragments of cDNA encoding the major house dust mite allergen Der p 1. *J Immunol* 1991;**147**:3768–3773.
- Ball T, et al. Isolation of an immunodominant IgE-hapten from an epitope expression cDNA library; dissection of the allergic effector reaction. *J Biol Chem* 1994;**269**:28323–28328.
- Van't-Hof W, Driedijk PC, Van den Berg M, Beck-Sickinger AG, Jung G, Aalberse RC. Epitope mapping of the *Dermatophagoides pteronyssinus* house dust mite major allergen Der p 2, using overlapping synthetic peptides. *Mol Immunol* 1991;**28**:1225–1232.
- Onk EK, Knox RB, Singh MB. Mapping of the antigenic and allergenic epitopes of Lol p VB using gene fragmentation. *Mol Immunol* 1995;**32**:295–302.
- Schneider T, et al. Human monoclonal or polyclonal antibodies recognize predominantly discontinuous epitopes on bee venom phospholipase A2. *J Allergy Clin Immunol* 1994;**94**:61–70.
- Vrtala S, et al. Conversion of the major birch pollen allergen, Bet v 1, into two non-anaphylactic T-cell epitope-containing fragments; candidates for a novel form of specific immunotherapy. *J Clin Invest* 1997;**99**:1673–1681.
- Zeiler T, et al. Recombinant allergen fragments as candidate preparations for allergen immunotherapy. *J Allergy Clin Immunol* 1997;**100**:721–727.
- Flicker S, et al. A human monoclonal IgE antibody defines a highly allergenic fragment of the major timothy grass pollen allergen, Phl p 5: molecular, immunological, and structural characterization of the epitope-containing domain. *J Immunol* 2000;**165**:3849–3859.
- 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* 1996;**271**:10967–10972.

37. Seiberler S, Scheiner O, Kraft D, Valenta R. Homology of two cDNAs coding for birch pollen allergens with calmodulin; protein bound Ca^{2+} affects the IgE-binding capacity. *Int Arch Allergy Immunol* 1992;**99**:380–381.
38. Seiberler S, Scheiner O, Kraft D, Lonsdale D, Valenta R. Characterization of a birch pollen allergen, Bet v III, representing a novel class of Ca^{2+} binding proteins; specific expression in mature pollen and dependence of patients IgE-binding on protein bound Ca^{2+} . *EMBO J* 1994;**13**:3481–3486.
39. Valenta R, et al. Calcium-binding allergens: from plants to man. *Int Arch Allergy Immunol* 1998;**117**:160–166.
40. Valenta R, Twardosz A, Swoboda I, Hayek B, Spitzauer S, Kraft D. Calcium-binding proteins in Type I allergy: elicitors and vaccines. In: Pochet R, Donato R, Haeich J, Heizmann C, Gehrke V, eds. Calcium: the molecular basis of calcium action in biology and medicine. Amsterdam: Kluwer Academic Press; 2000. p. 365–377.
41. Twardosz A, et al. Molecular characterization, expression in *Escherichia coli* and epitope analysis of a two EF-hand calcium-binding birch pollen allergen, Bet v 4. *Biochem Biophys Res Commun* 1997;**239**:197–204.
42. Engel E, et al. Immunological and biological properties of Bet v 4, a novel birch pollen allergen with two EF-hand calcium-binding domains. *J Biol Chem* 1997;**272**:28630–28637.
43. Hayek B, et al. Molecular and immunologic characterization of a highly cross-reactive two EF-hand calcium-binding alder pollen allergen, Aln g 4: structural basis for calcium-modulated IgE recognition. *J Immunol* 1998;**161**:7031–7039.
44. Niederberger V, et al. Calcium-dependent immunoglobulin E recognition of the apo- and calcium-bound form of a crossreactive two EF-hand timothy grass pollen allergen, Phl p 7. *FASEB J* 1999;**13**:843–856.
45. Laffer S, Vangelista L, Steinberger P, Kraft D, Pastore A, Valenta R. Molecular characterization of Bip 1, a monoclonal antibody that modulates IgE binding to birch pollen allergen, Bet v 1. *J Immunol* 1996;**157**:4953–4962.
46. Visco V, et al. Human IgG monoclonal antibodies that modulate the binding of specific IgE to birch pollen Bet v 1. *J Immunol* 1996;**157**:956–962.
47. Lebecque S, et al. Immunologic characterization of monoclonal antibodies which modulate human immunoglobulin E binding to the major birch pollen allergen Bet v 1. *J Allergy Clin Immunol* 1997;**99**:374–384.
48. Denepoux S, et al. Molecular characterization of human IgG monoclonal antibodies specific for the major birch pollen allergen Bet v 1. Anti-allergen IgG can enhance the anaphylactic reaction. *FEBS Lett* 2000;**465**:39–46.
49. Henderson LL, Larson JB, Gleich GJ. Maximal rise in IgE antibody following ragweed pollination season. *J Allergy Clin Immunol* 1975;**55**:10–15.
50. Heiss S, et al. Component-resolved diagnosis (CRD) of Type I allergy with recombinant grass and tree pollen allergens by skin testing. *J Invest Dermatol* 1999;**113**:830–837.
51. Yunginger JE, et al. Quantitative IgE antibody assays in allergic diseases. *J Allergy Clin Immunol* 2000;**105**:1077–1084.
52. Niederberg V, et al. The relationship between IgE binding capacity and biological activity of allergens: a study performed with purified recombinant pollen allergens. [Abstract]. *Allergy* 1999;**54**:Suppl 54:47.
53. van der Veen MJ, et al. Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J Allergy Clin Immunol* 1997;**100**:327–334.
54. van Hage Hamsten M, et al. Skin test evaluation of genetically engineered hypoallergenic derivatives of the major birch pollen allergen, bet v1: results obtained with a mix of two recombinant Bet v 1 fragments and recombinant Bet v 1 trimer in a Swedish population before the birch pollen season. *J Allergy Clin Immunol* 1999;**104**:969–977.
55. Pauli G, et al. Comparison of genetically engineered hypoallergenic rBet v 1 derivatives with rBet v 1 wild-type by skin prick and intradermal testing: results obtained in a French population. *Clin Exp Allergy* 2000;**30**:1076–1084.
56. Ortega E, Schweitzer-Stenner R, Pecht I. Possible orientational constraints determine secretory signals induced by aggregation of IgE receptors on mast cells. *EMBO J* 1988;**7**:4101–4109.
57. Syed RS, et al. Efficiency of signalling through cytokine receptors depends critically on receptor orientation. *Nature* 1998;**395**:511–516.
58. Field KA, Holowka D, Baird B. Compartmentalized activation of the high affinity immunoglobulin E receptor within membrane domains. *J Biol Chem* 1997;**272**:4276–4280.
59. Brown DA, London E. Functions of lipid rafts in biological membranes. *Annu Rev Cell Develop Biol* 1998;**14**:111–136.
60. Valenta R, et al. Genetically engineered and synthetic allergen derivatives: candidates for vaccination against Type I allergy. *Biol Chem* 1999;**380**:815–824.