

REVIEW

Allergens in rhinitis. B- and T-cell epitopes of allergen molecules*

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The allergens

The elicitation of rhinitic symptoms is provoked by molecules derived from a wide variety of sources. The primary sources are related to climatic conditions and living habits of the individual communities. In the northern temperate zone the most important sources are grasses of the *Poaceae* family, house dust mites of the *Dermatophagoidae* family, trees of the birch family *Betulaceae*, weeds of the *Asterales* order (like ragweed and *Artemisia*) and last but certainly not least, domestic mammals of which the house cat is the main culprit.

The various sources all contain a wide array of potentially allergenic molecules, some of which are of importance from a clinical point of view. Tables 1, 2 and 3 depict a selection of major allergens derived from main sources of the northern temperate zone. The allergen molecules have only a few characteristics in common, e.g. they are all proteins some of which are glycosylated and generally they have a molecular size ranging from 5000 to 40 000 Da. An important property is their ability to dissolve readily under conditions encountered on the human mucosal membranes.

During the last decade a lot of attention has been focused on the protein chemistry of major allergen molecules. The introduction of recombinant DNA (rDNA) techniques has allowed more detailed studies of the structure and variability of the allergens. Much of this work has been performed as a prelude to detailed studies of the interaction between the immune system and the invading allergen molecules.

The epitopes

Once introduced to the immune system the allergens elicit a B-cell response with ensuing antibody production which in the allergic patient is primarily of IgE class, i.e. some immunomodulation has taken place. The regulation is T-cell dependent. This in turn means that

the specificities of both antibodies and T-cell receptors (TCR) are involved in the development of allergic disease. B-cell epitopes are entities recognized by antibodies and T-cell epitopes are entities recognized by T-cell receptors in the context of Major Histocompatibility Complex (MHC) class II molecules (HLA DR/DP/DQ, see below).

B-cell epitopes are primarily three dimensional structures consisting of amino acids spacially arranged in a distinct configuration. Once the 3-D structure is destroyed, the forces providing the binding energy weaken and hence lower the affinity of the interaction between antibody and the corresponding epitope. Therefore, B-cell epitopes require a relatively intact molecular ambience to bind antibodies. The same tertiary constraints are not as important for T-cell epitopes in order to mediate the contact between the TCR on one hand and the HLA class II molecule on the other. Therefore, the identification of T-cell epitopes has been facilitated as short linear stretches of amino acids which can easily be synthesized chemically for direct use in epitope mapping. Such short peptides generally bind antibodies with only low affinity or hardly at all. B-cell epitopes usually need large stretches of peptide backbone to curl up in a conformation that can be recognized by antibodies elicited by the intact allergen molecule.

Studies of B-cell epitopes have in many cases been facilitated by the application of murine monoclonal antibodies (MoAbs). There is no prior reason to suspect that murine and human antibodies differ fundamentally in their ability to recognize allergen molecules. To make up for the lack of monoclonal allergen specific human IgE antibodies, several of the important studies of B-cell epitopes of allergen molecules have been performed with MoAbs. However, human B-cell crossreactivity between related allergens has been investigated by various IgE binding inhibition assays from which a lot of information has been derived. In some cases shorter synthetic peptides have been included in the definition of IgE binding epitopes. More specific data could potentially be obtained with the access to monoclonal human IgE. This may be possible in the near future with the advent of recombinant antibodies.

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Table 1. Major allergens used for epitope studies. House dust mites and mammals

Allergen source	Allergen	Mol.wt. (kDa) SDS-PAGE
House dust mites (<i>Dermatophagoides</i>):		
<i>D. pteronyssinus</i>	<i>Der p I</i>	24
	<i>Der p II</i>	15
<i>D. farinae</i>	<i>Der f I</i>	24
	<i>Der f II</i>	15
	<i>Der f III</i>	29
<i>D. microceras</i>	<i>Der m I</i>	24
Mammals		
<i>Felis domesticus</i> (cat)	<i>Fel d I</i>	17

Genetics of allergy

Allergic disease has got hereditary components. However, the nature of this is still a matter of controversy. Several groups are currently investigating the cognate response, i.e. specific recognition as well as the non-cognate, i.e. an overall propensity for high IgE production. These aspects are outside the scope of the present paper and interested readers are referred to selected references [1–3].

Epitope presentation

Exposure to the allergen sources is an important step in the development of allergic disease, i.e. which molecules expose the immune system of the potential allergic patient at different stages of life. The initial steps of sensitization have not been described in detail as yet, but the consensus is that the allergic immune response requires T-cell regulation. This means that antigen presenting cells (APCs) are likely to be the initiators of

Table 2. Major allergens used for epitope studies. Trees

Allergen source	Allergen	Mol.wt. (kd) SDS-PAGE
<i>Fagales</i>		
<i>Quercus alba</i> (white oak)	<i>Que a I</i>	17
<i>Betula verrucosa</i> (birch)	<i>Bet v I</i>	17
<i>Alnus glutinosa</i> (alder)	<i>Aln g I</i>	17
<i>Carpinus betulus</i> (hornbeam)	<i>Car b I</i>	17
<i>Corylus avelana</i> (hazel)	<i>Cor a I</i>	17

Table 3. Grass pollen allergen groups used for epitope studies

Group of grass allergens	Mol.wt. (kDa) SDS-PAGE	pI	IgE binding prevalence %	Best described allergen
Group I	30–35	5–6	90–100	<i>Lol p I</i>
Group II	11	5	90–100	<i>Lol p II</i>
Group III	11	9	90–100	<i>Lol p III</i>
Group IV	50–60	9–10	40	<i>Lol p IV</i>
Group V	25–32	4–5	90–100	<i>Phl p V</i>

the ensuing allergic response. The APCs are cells of the reticulo-endothelial lineage, e.g. Langerhans cells of the skin and follicular dendritic cells of lymphnodes. By endocytosis they take up incoming material like allergenic protein molecules and break down these molecules enzymatically. The fragmented allergens will bind HLA class II molecules which line the luminal side of fused vacuoles inside the APC. The class II molecules carrying the short stretches of allergen, typically peptides of 12–17 amino acids, will be exposed on the cell surface after the vesicle has fused with the cell membrane.

The allergen derived peptide embedded in the bilabial groove of the class II molecules is the activator of T-cells, i.e. this complex conveys information to the immune system on the allergen exposure. The specificity of the elicited T-cell response is a direct consequence of the peptide/class II combination which means that the signal activating the T-cell is really a combination of a specific amino acid sequence (the epitope) complexed with a particular class II molecule. However, although class II molecules restrict the presentation of particular T-cell epitopes, several studies have shown that degeneracy occurs in the combination of certain epitopes and class II molecules.

The functional outcome of the selective T-cell activation and subsequent proliferation is dependent on the phenotype of the individual T-cell, i.e. which secreted cytokines dominate the response to activation. TH-1-like cells will primarily secrete IFN- γ and IL-2 whereas TH-2-like cells will produce large quantities of IL-4 and IL-5. Studies of allergen specific T-cell clones indicate that atopic persons respond to allergen molecules with a TH-2-like pattern. Non-atopic persons exhibit a TH-1-like response.

The following paragraphs on B- and T-cell epitopes of major allergen molecules known to be involved in rhinitis are not exhaustive reviews. They are intended to demonstrate the scope of such studies and to present some of

the most important results from the author's point of view.

Epitopes of grass pollen allergens

The major grass pollen allergens are the group 1 and group 5 allergens. The group 1 allergens have been defined in most of the grasses investigated from both the temperate and the subtropical zones. B-cell epitopes are widely shared among group 1 allergens of different species of grasses as has been defined by MoAbs. This is supported by the clinical observation that grass allergic patients react with several species, including some to which the individual patient has never been exposed. The group 5 allergens are apparently not as taxonomically widespread as group 1 but to a large extent they share B-cell epitopes as demonstrated by both MoAbs and IgE [4-10].

Epitopes of tree pollen allergens

The *Fagales* order includes trees of the birch family. Those members of this taxonomic entity which have been immunochemically analysed all exhibit one major allergen, the group 1 as represented by *Bet v 1* of birch (*Betula verrucosa*), *Aln g 1* from alder (*Alnus glutinosa*), *Cor a 1* from hazel (*Corylus avellana*) and *Car b 1* from hornbeam (*Carpinus betulus*). The B-cell epitope pattern of these allergens have been analysed for shared B-cell epitopes by MoAbs [Dr H. Ipsen, personal communication] and by IgE absorption [11]. In concordance with clinical findings the allergens were shown to be highly crossreactive. An indication of the tertiary constraints of B-cell epitopes is the destruction of almost all antibody binding capacity of *Bet v 1* by enzymatic removal of the 20 C-terminal amino acids [Dr H. Ipsen, personal communication].

Recently, evidence has appeared that the allergens are represented by several isoforms, i.e. variants that differ by just a few amino acid residues of their primary sequence. As revealed by two-dimensional electrophoresis of birch pollen extract, *Bet v 1* appears as approximately 20 individual protein spots. These spots all exhibit IgE binding epitopes and furthermore they showed individual epitope structures as determined by MoAbs. An interesting aspect of this individuality is that each birch allergic patient seems to have his own specific recognition pattern with regard to IgE binding. This observation is likely to have important implications for the application of recombinant allergens in future therapy, i.e. which isoform should be used? As far as T-cell epitopes are concerned experiments with cloned variants of *Bet v 1* indicate that also T-cell epitope

patterns vary between isoforms of the allergen [Dr S. Sparholt, personal communication]. Some T-cell epitopes are shared between *Bet v 1*, *Cor a 1* and *Aln g 1* as demonstrated by Ebner *et al.* [12]. With regard to the localization of the individual T-cell epitopes along the primary sequence of *Bet v 1* both Ebner *et al.* [13,14] and Drs S. Sparholt and J. Nedergaard Larsen [personal communication] have identified at least 10 epitopes. These were scattered along the entire sequence and each patient tested exhibited individual epitope recognition patterns.

Epitopes of house dust mite allergens

The two major groups of allergens from house dust mites (HDM), i.e. group 1 and 2 are very important from a clinical point of view and have received due attention over the years.

Der p 1 (having 222 amino acids encoded by a single gene) from *Dermatophagoides pteronyssinus* have been analysed for B-cell epitopes by means of short synthetic peptides as well as larger fragments expressed from rDNA constructs. Recombinant fragments p53-99 and p98-140 were shown to bind IgE from house dust mite allergic patients [15] so these stretches of polypeptide apparently fold up in partially correct configurations. A different approach demonstrated that rabbit antibodies to synthetic peptides p52-71 and p117-133 bound the intact *Der p 1* and furthermore bound *Der f 1* from closely related *D. farinae* [16]. By means of MoAbs and human IgE [17] an extensive range of common, as well as species specific, B-cell epitopes have been demonstrated on *Der p 1*, *Der f 1* and *Der m 1* (from *D. microceras* which is *D. farinae*'s sibling species). These observations are in line with clinical observations that the majority of HDM allergic patients react with more than one species.

The T-cell epitope pattern exhibited by *Der p 1* has been studied at the polyclonal as well as the monoclonal level. O'Brien *et al.* [18] investigated the polyclonal T-cell response of 35 HDM-sensitive individuals. rDNA techniques were applied to produce seven fragments comprising from 39 to 114 amino acids derived from the corresponding DNA sequence. All fragments encompassed T-cell epitopes but 12 of 18 responsive patients reacted primarily with epitopes within the 56 N-terminal amino acids. Two patients reacted with epitopes of the C-terminal half. Yssel *et al.* [19] used rDNA techniques to map the minimal epitopes of *Der p 1* that activated cloned T-cells from two HDM-sensitive patients. The epitopes found were: p45-67, p94-104 and p117-143, i.e. scattered along the primary sequence. The corresponding HLA class II restriction pattern was degenerate. By means of synthetic peptides Higgins *et al.* [20]

demonstrated that a particular HDM-allergic patient responded to several overlapping T-cell epitopes in the region p101-143 and that HLA class II restriction to these epitopes exhibits heterogeneity.

B-cell epitopes of *Der p* II (129 amino acids encoded by a single gene) has been investigated by rDNA techniques [21]. Large overlapping fragments of the allergen (p1-69, p42-117 and p69-129) expressed as deletion mutants fused to the glutathione transferase of pGEX vectors hardly bound any IgE from house dust mite allergics. This suggests that the B-cell epitopes of *Der p* II are highly conformational.

T-cell epitopes of *Der p* II have been investigated by overlapping synthetic peptides [22]. Polyclonal *Der p* II specific T-cell lines derived from five house dust mite allergic patients reacted with at least 10 different epitopes scattered along the entire allergen molecule. Interestingly, each donor exhibited an individual epitope reactivity pattern. T-cell clones obtained from a single patient all reactive with p116-129 used different TCR V-beta gene segments. This indicates a certain degree of degeneracy in the HLA class II/epitope/TCR binding. In a study by O'Hehir *et al.* [23] who compared the T-cell epitope recognition of HDM allergics and non-atopic control individuals, it was shown that T-cell epitopes are present in all regions of *Der p* II. Among the 18 persons tested it seemed that the majority (atopics as well as non-atopics) responded to T-cell epitopes in the regions p11-50 and p61-104.

Epitopes of mammalian allergens

The major cat dander allergen *Felis domesticus* (*Fel d*) I (a two-chain structure encoded by separate genes) accounts for the reactivity of approximately 30% of the T-cells cloned from cat allergic patients [24]. This indicates that although the molecule carry more than half of the IgE binding capacity of a cat hair and dander extract it has less than a third of the T-cell stimulating activity. The corresponding T-cell epitopes were mapped with overlapping synthetic peptides covering the entire sequence of both polypeptide chains. Epitopes implicated were: chain 1 p39-52 and p53-66; chain 2 p9-21, p22-35 and p57-70. The patients exhibited individual T-cell epitope recognition patterns. Other studies have identified at least two additional T-cell epitopes on chain 2 one of which overlaps an IgE binding epitope.

Where do we stand?

As described above both B-cell and T-cell epitopes have been identified on the major allergens from several of the clinically most important allergen sources. The

information derived has increased our knowledge of the interaction between allergen molecules and the immune system. We have reached a stage where the epitopes can be applied directly as reagents in the detailed study of the basic mechanisms of the allergic immune response. With the information on epitopes in hand we can specifically address the basic question of why some people become allergic and others do not. Another important issue to be addressed is: how can the allergic immune response be redirected towards a non-allergic behaviour?

A few things should be kept in mind:

- 1 B-cell epitopes usually require three-dimensional 'intactness' to be recognized by antibodies.
- 2 T-cell epitopes do not have the same conformational constraints as B-cell epitopes to be recognized by the T-cell receptor.
- 3 All allergens studied display multiple T-cell epitopes.
- 4 Major allergens are found in many iso-forms that display differences in epitope structure.
- 5 T-cell epitopes are scattered along the entire amino acid sequence of all allergens studied.
- 6 T-cell epitope recognition pattern varies from one patient to the other.
- 7 Some T-cell epitopes can be recognized in the context of several HLA class II molecules and some T-cell epitopes can bind several TCR V-beta chains.
- 8 Almost all allergic patients react with several allergen molecules from each individual source.

The present specific allergy treatment, i.e. hyposensitization (or SIT) based on standardized allergen extracts containing all important allergens is a very effective means of treatment of rhinitis [25]. Furthermore, SIT has been shown to influence the exacerbation and development of allergic disease [26]. It is the hope for the future that the information on epitopes of allergens can be used to further improve the effectiveness of diagnosis and specific treatment of allergic diseases.

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