Allergy 2001: 56: 478–490
Printed in UK. All rights reserved

ALLERGY
ISSN 0105-4538

Review article

Cross-reactivity of IgE antibodies to allergens

The cross-reactivity of IgE antibodies is of interest for various reasons, three of which are discussed. Firstly, from the clinical view, it is important to know the patterns of cross-reactivity, because they often (but not always) reflect the pattern of clinical sensitivities. We discuss the cross-reactivities associated with sensitization to pollen and vegetable foods: PR-10 (Bet v 1-related), profilin, the cross-reactive carbohydrate determinant (CCD), the recently described isoflavone reductase, and the (still elusive) mugwort allergen that is associated with celery anaphylaxis; cross-reactivities between allergens from invertebrates, particularly tropomyosin, paramyosin, and glutathione S-transferase (GST); and latex-associated cross-reactivities. Clustering cross-reactive allergens may simplify diagnostic procedures and therapeutic regimens. Secondly, IgE cross-reactivity is of interest for its immunologic basis, particularly in relation to the regulation of allergic sensitization: are IgE antibodies to allergens more often cross-reactive than IgG antibodies to "normal" antigens? If so, why? For this discussion, it is relevant to compare not only the structural relation between the two allergens in question, but also the relatedness to the human equivalent (if any) and how the latter influences the immune repertoire. Thirdly, prediction of IgE cross-reactivity is of interest in relation to allergic reactivity to novel foods. Cross-reactivity is a property defined by individual antibodies to individual allergens. Quantitative information (including relative affinity) is required on cross-reactivity in the allergic population and with specific allergens (rather than with whole extracts). Such information is still scarce, but with the increasing availability of purified (usually recombinant) allergens, such quantitative information will soon start to accumulate. It is expected that similarity in short stretches of the linear amino-acid sequence is unlikely to result in relevant cross-reactivity between two proteins unless there is similarity in the protein fold.

R. C. Aalberse, J. H. Akkerdaas, R. van Ree

CLB and Laboratory for Experimental and Clinical Immunology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

Key words: allergen structure; allergenicity; cross-reactive carbohydrate determinant (CCD); cross-reactivity.

Prof. R. C. Aalberse
CLB, Department of Immunopathology
Plesmanlaan 125
1066 CX Amsterdam
The Netherlands

Accepted for publication 5 February 2001

A short history of allergen cross-reactivity

Allergen cross-reactivity works usually, but not always, "according to the book": in general, cross-reactivity reflects the phylogenetic relations between organisms. A phylogenetic relation results in a high degree of homology in the primary structure of the proteins (the amino-acid sequence; see Table 1 for the database accession codes of the proteins that will be discussed). High homology in the primary sequence results in homologous 3-D structures and thus, potentially, in cross-reactivity. Serum albumins from vertebrates are often cross-reactive (1). Homologous allergens from phylogenetically related grasses tend to be cross-reactive (2), and IgE antibodies to allergens from peanuts are often cross-reactive with homologous proteins in soybean and other legumes.

However, cross-reactivities between much more distantly related organisms have been known for some time. Some of the highlights in the history of unexpected cross-reactions in allergy are ragweed/banana, which was recognized as early as 1970 (3), birch/apple (4), cross-reacting carbohydrate determinant (5), profilin (6, 7), mugwort/celergy (8), latex/banana/avocado (9), and mite/snail/shrimp (10–14).

The birch-apple story started in 1977 when Hannuksela & Lahti (4) described the association on clinical grounds. For two independent reasons, cross-reactivity was not easily demonstrable at that time. Firstly, the apple extracts that were then available were deficient in the relevant proteins. Only when Björkstén et al. adapted an extraction procedure (15) could suitable extracts be prepared. Secondly, the apple extracts contained cross-reacting structures (CCDs [5]), but these did not show well in the technique that was used at that time: crossed radioimmunoelectrophoresis (16, 17). This changed dramatically in the early 1980s when immunoblotting became the preferred procedure. This resulted in the recognition of two types of cross-reactivity, one due to proteins and the other due to sugars (glycans on glycoproteins); the latter type was

called cross-reacting carbohydrate determinant (CCD) (5). Soon it became clear that, in addition to CCD-type cross-reactivity, there had to be at least two proteins involved in the birch-apple cross-reactivity, one being the major birch-pollen allergen and the other an elusive, slightly smaller protein that was even more broadly cross-reactive, as with grass pollen (18). This protein was identified by molecular cloning and proved to be the proline-binding protein, profilin (6). Thus, the molecular-biology approach not only led to the identification of this most important cross-reacting allergen, but it also provided an efficient purification procedure: affinity chromatography with polyproline.

Two more allergens have recently been added to this list of pollen-associated food allergens: one is the homolog of the sweet-tasting 31-kDa protein thaumatin (PR-5), which has been described in apple (19), cherry (20), and strawberry among other sources. The other is isoflavone reductase (21), a 33–35-kDa protein.

The mugwort-celery connection (8, 22) has been investigated extensively by Wüthrich and coworkers (23–28) and Vallier et al. (29, 30). The molecular basis for this association between mugwort pollen sensitivity and anaphylactic reactivity to a heat-stable allergen in celery (24, 28, 29, 31–33) is still unresolved. Part of the serologic cross-reactivity is due to profilin (30) and CCD, but this does not explain the striking clinical phenotype. Mugwort pollen does not contain the PR-10 protein (a Bet v 1 homolog), and so this is not a candidate either (34). One potential candidate is a lipid transfer protein (LTP) (see below) (35). Another candidate is the major allergen Art v 1 (36), which is heavily glycosylated. This may hamper the expression of an immunologically fully reactive (eagerly awaited) recombinant protein that might be used for analyzing this intriguing and clinically highly relevant cross-reactive system.

LTPs, which are also classified as PR proteins (PR-14) (37), are small, very stable, proteins. They have been identified as the major cause of anaphylactic sensitivity to fruits (38–40). Cross-reactivity has been found among LTPs from Rosaceae species (apple/almond/peach). Based on the amino-acid identity data (less than 35% identity between LTP from mugwort and LTP from *Parietaria*), cross-reactivity among LTPs is likely to be lower than among, for example, profilins. Our own preliminary data suggest significant cross-reactivity between LTPs from celery and apple.

The relations within the latex/fruit cluster (9, 37, 41, 42) are even more complex than in the previous clusters. Exposure to rubber latex occurs by at least three routes: via the airways (as dust mostly from gloves in medical settings), via the skin, and parenterally during operations and other procedures. In each of these three situations, the allergens involved may differ. Reactivity to foods associated with latex sensitivity, such as avocado and banana, occurs with very different prevalences. Last but not least, from the point of view of a protein chemist, rubber latex is a more challenging biologic fluid than a pollen extract.

Chitinase-related proteins (the basic 25–35-kDa variants, PR-3 and PR-4 [37]) are a major source of cross-reactivity between rubber latex (Heb v 6 [43]) and fruits such as banana, avocado (Pers a 1 [44]), and chestnut. Hevein, which is the smaller chitin-binding N-terminal domain in the precursor, is easily severed. It appears to be the major cross-reactive part (45). The remaining part of the precursor protein is related to the wound-induced proteins (Win-proteins). These are also well conserved, but do not appear to contribute to cross-reactivity. Because these enzymes often cleave not only chitin (poly-*N*-acetylglucosamine) but also a structurally related bacterial cell-wall polymer, which consists of *N*-acetylglucosamine and *N*-acetylmuramic acid, they are also referred to as lysozymes. Another chitinase-related protein in rubber latex, hevamin, does not appear to be important in cross-reactivity.

In addition to the chitinases, rubber latex contains a number of other proteins that may to some degree contribute to cross-reactivity. There is a discrepancy regarding the cross-reactivity of Hev b 7, a 46-kDa protein (46) related to the potato allergen patatin (47, 48). Seppälä et al. (49) reported cross-reactivity in 10/35 adult patients (but not in children). However, in a similar patient population, no cross-reactivity was found by Sowka et al. (50).

Invertebrate allergens

In addition to the above-mentioned examples of cross-reactivity between plant-derived proteins, there are some examples of cross-reactivity between allergens from invertebrates, particularly between mite and snail (11, 12); mite, shrimp, and cockroach (13); mite and caddis fly (51); and mites and schistosomes. Three of the proteins that are involved in these examples are tropomyosin, paramyosin (52), and glutathione S-transferase (GST).

The CCD structure (or, rather, structures, because this is really a family rather than one specific structure) has been largely determined, both chemically (53–70) and in 3-D (71, 72) (Fig. 1). This structural information refers to the N-linked glycans. One important unresolved question is whether and how O-linked sugars fit into this picture. Another, even more important, question is the relation (or, rather, lack of relation) between IgE binding and biologic symptoms (73). In most cases, the biologic activity is low compared to the immunochemical activity, but in several cases biologic activity was not low at all (74). In this context, the data on the sea-squirt glycan are also of interest (75–78). One possible factor might be the source of the glycan used for testing. If the glycan is derived from a source material that caused symptoms in the patients, the

Table 1. Entry codes for proteins referred to in figures. Sequences are from Swiss Prot database (sp) or from trEMBL database (tr). These can be accessed at http://www.expasy.ch

Amb e 1	sp	P27759
Avocado chitinase	tr	P93680
Banana glucanase	tr	O22317
Banana pectate lyase	tr	Q9SDW4
Barley group 5	tr	O04828
Bet v 1	sp	P15494
Bet v 4	tr	O82040
Birch chitinase	tr	Q9M3T4
Birch isoflavone reductase	tr	O65002
Birch profilin	sp	P25816
Bovine albumin	sp	P02769
Bovine lactoglobulin	sp	P02754
Brazil nut 2S	sp	P04403
Cauliflower LTP	sp	Q42641
Cedar Cry j 1	sp	P18632
Chitinase avocado	tr	P93680
Chitinase potato	sp	P52403
Cladosporium enolase	sp	P42040
Cod parvalbumin	sp	P02622
Cucumber patatin	tr	O23784
Cyn d 1	tr	O04701
Cyn d profilin	sp	O04725
Dau c 1	sp	O04298
Der f 1	sp	P16311
Der f 2	sp	Q00855
Der p 1	sp	P08176
Der p 2	sp	P49278
Dog serum albumin	tr	Q9TSZ4
Equ c 1	sp	Q95182
Extensin	sp	Q03211
Fruit fly paramyosin	tr	O18392
Grapefruit isoflavone reductase	tr	O49820
Hev b 7	tr	O65811
Hevea chitinase	sp	P23472
Hevea enolase	tr	Q9LEJ0
Hevea glucanase (Hev b 2)	sp	P52407
Hevein avocado	tr	P93680
Hevein potato	sp	P52403
Horse Equ c 1	sp	Q95182
Human A1micro	sp	P02760
Human albumin	sp	P02768
Human calmodulin	sp	P27482
Human cathepsin K	sp	P43235
Human enolase	sp	P06733
Human GST	sp	P46439
Human myosin	tr	Q9Y622
Human parvalbumin	sp	P20472
Human profilin	sp	P35080
Human serpin SCCI	sp	P29508
Human tropomyosin	sp	P06753
Jun a 3	sp	P81295
Jun o 2	tr	O64943
Jun o 2	tr	O64943
Lol p 1	sp	P14946
Lol p 5	sp	Q40240
Maize vicilin	tr	Q03865
Mal d 1	tr	Q43549
Mite GST (Der p 8)	sp	P46419
Mite tropomyosin	tr	O16188
MnSOD Asp	tr	Q9SM64
MnSOD Human	sp	P04179
MnSOD Peach	tr	Q9P945
Mustard 2S	sp	P15322
Ole e 1	sp	P19963
Orange glucanase	tr	O23783
Ovalbumin	sp	P01012
Par j 1	sp	P43217
Peach LTP	sp	P81402
Peanut vicilin (Ara h 1)	sp	P43237
Pear isoflavone reductase	tr	O81355
Phl p 1	sp	P43213
Phl p 5	tr	O81341
Phl p 7	tr	O04131
Pig serpin	sp	P80229
Potato patatin	sp	P15478
Rat n 1	sp	P02761
Salmon parvalbumin	sp	Q91483
Schisto GST	sp	P35661
Schisto paramyosin	sp	P06198
Sesame 2S	tr	Q9XHP1
Shrimp tropomyosin	sp	Q25456
Soy vicilin	tr	O22121
Strawberry thaumatin	tr	Q9SBT2
Tomato calmodulin	sp	P27161
Tyr p 2	sp	O02380

chance of a biologic effect seems to be higher. This suggests that there may be some subtle differences in structure that are not obvious with the current (immuno-) chemical analyses. It is likely that other families of glycans exist with similarly broad cross-reactivity patterns – for example, mold/yeast glycoconjugates.

This historical survey beautifully illustrates the need for close collaboration between clinicians and the laboratory. The observation of an association between sensitivities to allergens is an important starting point. The distinction between cosensitization and cross-reactivity requires *in vitro* experiments. Characterization of the cross-reacting component by immunoblotting, protein fractionation, and recombinant DNA technology requires feedback from the clinician, because some allergens that seem to be important in the laboratory prove to be (almost) without clinical relevance. If a patient is clinically reactive to two allergen source materials and the patient's serum contains a strongly cross-reactive antibody, it is tempting to assume that these two observations are causally related. This assumption has quite often proved to be wrong (if so, the confusion is usually due to the coincidental presence of IgE to CCD, which shows strongly on immunoblots, but has little *in vivo* activity).

Diagnostic and therapeutic issues

If two allergens are very similar, it does not increase the diagnostic accuracy to include both in the diagnostic test panel (79, 80). Similarly, successful treatment with one allergen is likely to relieve symptoms the other allergen as well (81). Particularly in the therapeutic situation, it is likely to be relevant to identify the primary sensitizing allergen, as this will cover the widest spectrum of specificities. Such identification can be made by *in vitro* reciprocal inhibition tests. Cross-

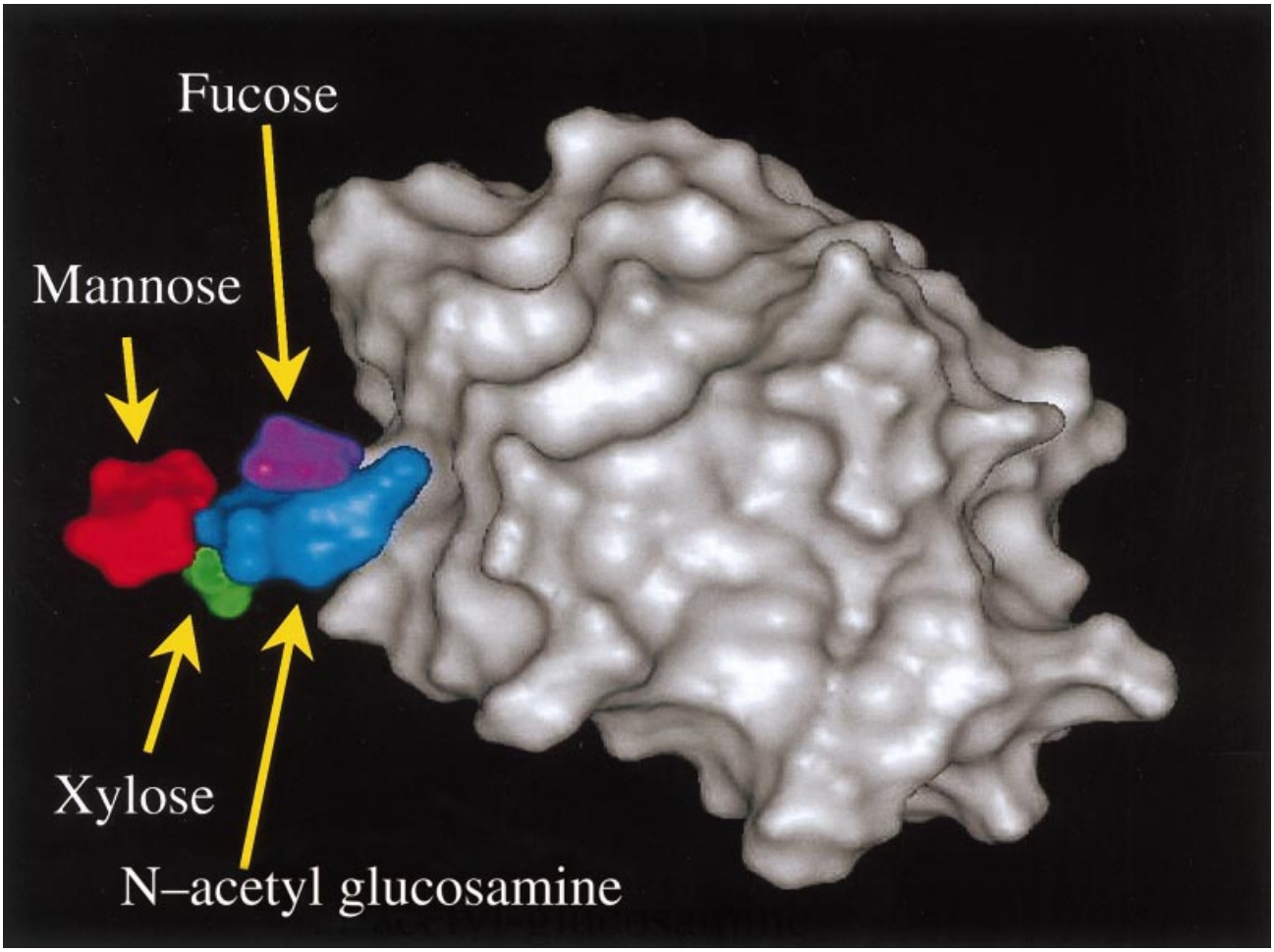

Figure 1. 3-D structure of cross-reactive carbohydrate determinant (CCD) (structures in color) shown as glycan attached to its parent protein (here erythrina lectin, the gray structure) (71–72). Two sets of atomic coordinates are available from Protein Data Bank (PDB, http://www.rcsb.org/pdb/searchlite.html): PDB1LTE.ENT (shown here) and PDB2MYR.ENT, with very similar 3-D structure for glycan.

reactivity *in vitro* is not, however, always reflected by cross-reactivity *in vivo*. As mentioned before, this is particularly true for CCD-type cross-reactivity. However, such discrepancies have been found also for peptidic cross-reactivity; for example, for some patients with *in vitro* IgE reactivity to profilins, no corresponding skin reactivity is found (82, 82). This may reflect low affinity and/or restricted epitope recognition.

Immunologic sensitization in relation to cross-reactivity

Most allergic patients react to more than a single allergen. To a large degree, this reflects multiple sensitization events as a consequence of the increased immunologic responsiveness present in atopy. These events are not fully independent, as a Th2 response to one allergen is likely to facilitate subsequent Th2 responses by creating and maintaining for some time a local milieu that favors Th2 responses. In this context, the Th2-promoting potential of effector cells in the allergic cascade, particularly mast cells and basophils, is obviously of interest. This "IgE breeds IgE" (84) phenomenon and the related phenomenon of epitope spreading (85) are not as strong as sometimes suggested. It is of note that even for allergens that are simultaneously presented to the immune system, such as cat allergen and mite allergen on dust particles, cosensitization is not universal.

An alternative cause of allergic multireactivity is cross-reactivity. The definition of cross-reactivity is based on immunologic recognition. Two allergens are cross-reactive if there is a single antibody (or T-cell receptor) that reacts with both. This basic definition can be embellished by defining an affinity threshold and by including something about clinical relevance. It is important to appreciate that it may be possible to show convincingly that allergens are cross-reactive, but that it is impossible to prove that allergens are *not* cross-reactive. Allergens are non-cross-reactive until proven otherwise, and exhaustive testing is practically impossible.

In general terms, repeated contact with "the

allergen'' is a prerequisite for allergic reactions. The more accurate description is, of course, repeated contact with substances that have ''some common structural feature''. For this discussion, three points in time and three cells have to be distinguished. The first point in time is the immunologic priming. At this stage, the allergen has to be recognized by virgin T and B cells. The second point in time is the contact of the allergen with memory T and B cells. The third point in time is the contact between the allergen and the sensitized mast cell.

These three events are usually triggered by an identical allergen. How much similarity is required between the ''allergens'' on these three occasions? What happens if the allergens are only distantly related?

Cross-reactivity at the B-cell level

As discussed in more detail elsewhere (86), the surface structure recognized by antibodies is usually shaped by 6–10 amino acids, some of which are neighbors also at the primary structure level (the amino-acid sequence). A substantial contribution to the epitope usually comes from amino acids that are further apart in the linear sequence. Classically, antibodies that are reactive with isolated peptides of fewer than 15 amino acids are assumed to be directed to a linear epitope, whereas antibodies that do not react with small peptides from the linear sequence are classified as being directed to a conformational epitope. It must be stressed, however, that it is highly exceptional to find a small peptide that closely mimics the intact protein in its interaction with an antibody. There are at least two reasons why the interaction of an antibody with a linear peptide is usually several orders of magnitude weaker than with the full protein. The first is that the peptide represents only part of the epitope. The second reason is that the peptide is much more flexible than the complete protein. While such flexibility may allow the peptide to adapt its structure to an optimal fit, it decreases overall affinity because of the loss of entropy upon binding.

High-affinity cross-reactivity between proteins is thus likely to require, in general, a similar protein fold (or, rather, protein domain fold). Grafting a linear amino-acid sequence into an unrelated protein (excluding the C- or N-terminal ends) is not known to result in relevant cross-reactivity. No well-characterized example is known of high-affinity cross-reactivity between two proteins with a different fold, but with a short internal sequence of amino acids that are identical. The most probable situation where this might occur would be when the identical amino acids are either the C- or the N-terminal part in both proteins.

Many examples of single-point mutations are known that affect antibody binding drastically. This does not necessarily mean that this amino acid is the main contact residue, as a single mutation may change a much wider surface area. The interaction between antibody and antigen obviously occurs via their surfaces. This does not mean that structures below the surface are irrelevant: mutations in noncontact residues can result in a change in the configuration of contact residues and thus in a change in the affinity.

The effects described above may be quite specific for one particular antibody. On a global level, it should be obvious that if two allergens are cross-reactive when tested with one antibody, they need not be cross-reactive when tested with the next antibody. Yet, it is not always appreciated, for example, that not all IgE antibodies to profilin are cross-reactive: some react with grass profilin, and not with birch profilin (87). If a mutation affects one antibody much more than another antibody, it is tempting, but incorrect, to conclude that these two antibodies are directed to different epitopes. Similarly, if one antibody is cross-reactive and another antibody is not, these antibodies may still react with the same surface patch.

Cross-reactivity at the T-cell level

The marked difference in antigen recognition by the T-cell receptor (TCR) compared to that by antibodies obviously has implications for cross-reactivity. T-cell reactivity may be as much affected as antibody reactivity by a single amino-acid substitution. However, because of the smaller size of the peptide recognized by the T-cell receptor, the T cell is, for statistical reasons, more likely to be confronted with indistinguishable structures originating from different proteins. Moreover, because T cells are not (or, hardly) undergoing somatic mutation and affinity maturation, T-cell specificity is less adaptive than B-cell specificity. Antigen contact influences various T-cell characteristics, but not the intrinsic affinity of the TCR for the antigen. This is a major difference from antibody recognition: antibodies need to fit the antigen tightly in order to survive the second round of selection during affinity maturation.

Cross-reactivity at the mast-cell level

Both B cells and mast cells depend on antigen recognition via antibodies. Yet, cross-reactivity at the mast-cell level is distinct from that at the B-cell level (86). One important difference is the need of a B cell for additional triggers. This is in clear contrast to the more modest requirements of a mast cell. Mast-cell triggering can be modulated by various factors (adhesion molecules, cytokines, or chemokines), but these are not as essential for triggering a mast cell as

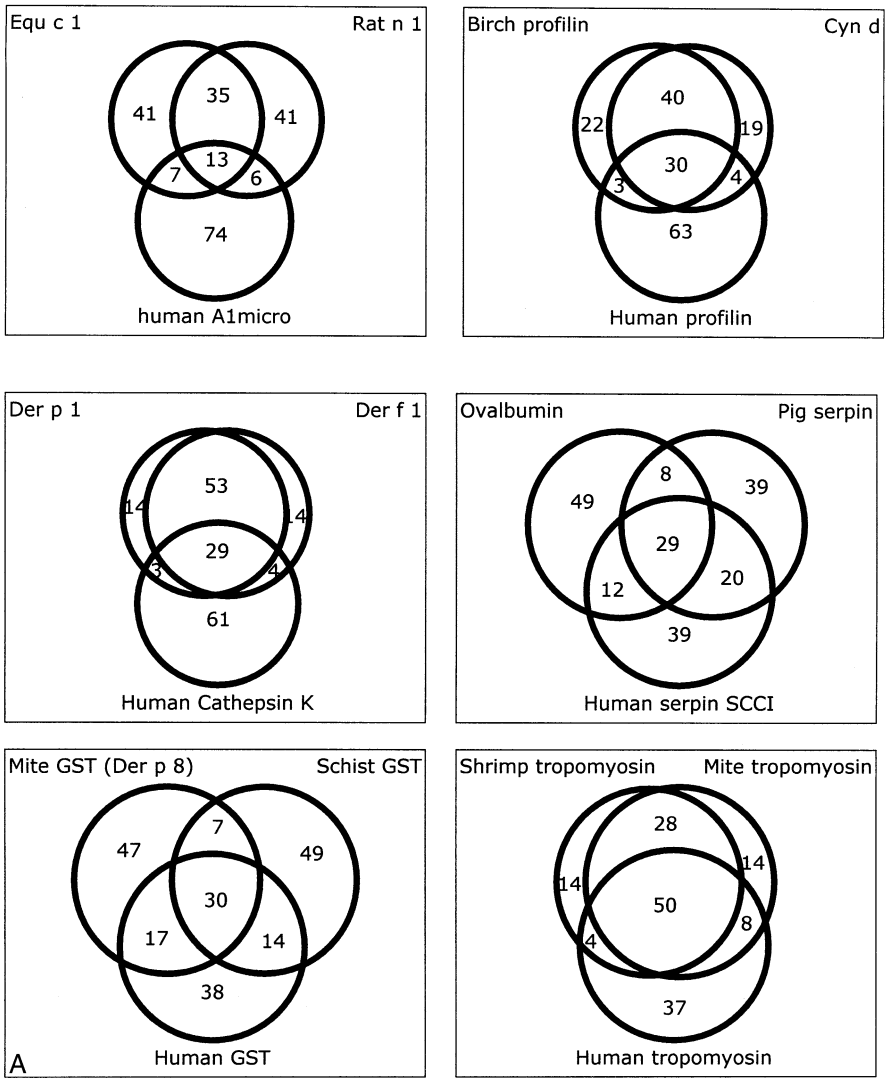

Figure 2A and B. Amino-acid identity between triplets of proteins, one of which is human protein in each triplet.

they are for triggering a B cell. Moreover, the antibodies on a B-cell surface are monoclonal, whereas the antibodies on the mast-cell surface are polyclonal. The implications for cross-linking, which is the basis for activation of both B cells and mast cells, have been discussed elsewhere (86, 88).

In many cases of symptomatic cross-reactivity between allergens, it is likely that the immune system has been triggered by only one of the cross-reacting partners. The other partner reacts with cell-bound antibodies on the mast cells near the mucosal surface, but does not reach the immune system itself.

Cross-reactive priming

If the immune system has access to both cross-reactive partners, how does the first contact influence the reaction of the immune system to a subsequent contact with the cross-reactive partner? In some cases, the first contact has a tolerizing effect, as in the case of autologous proteins (Fig. 2). In this situation, part of the potential immune reactivity is downregulated or deleted; therefore, the immune response to an extraneous protein with immunologic similarity to an autologous protein is likely to be weaker than that to a completely foreign protein. If, on the other hand, the first interaction resulted in the generation of memory cells that recognize the cross-reacting antigen, an enhancing effect might be expected. This positive effect, cross-reactive priming, is more likely to occur for T cells than for B cells (see below). For T cells, this effect has been most clearly demonstrated in a classical, artificial system: carrier priming. An antihapten immune response following immunization with a hapten-carrier complex is stronger if an experimental animal has been pre-immunized with the carrier protein (89). This type of cross-reactive priming does not necessarily result in

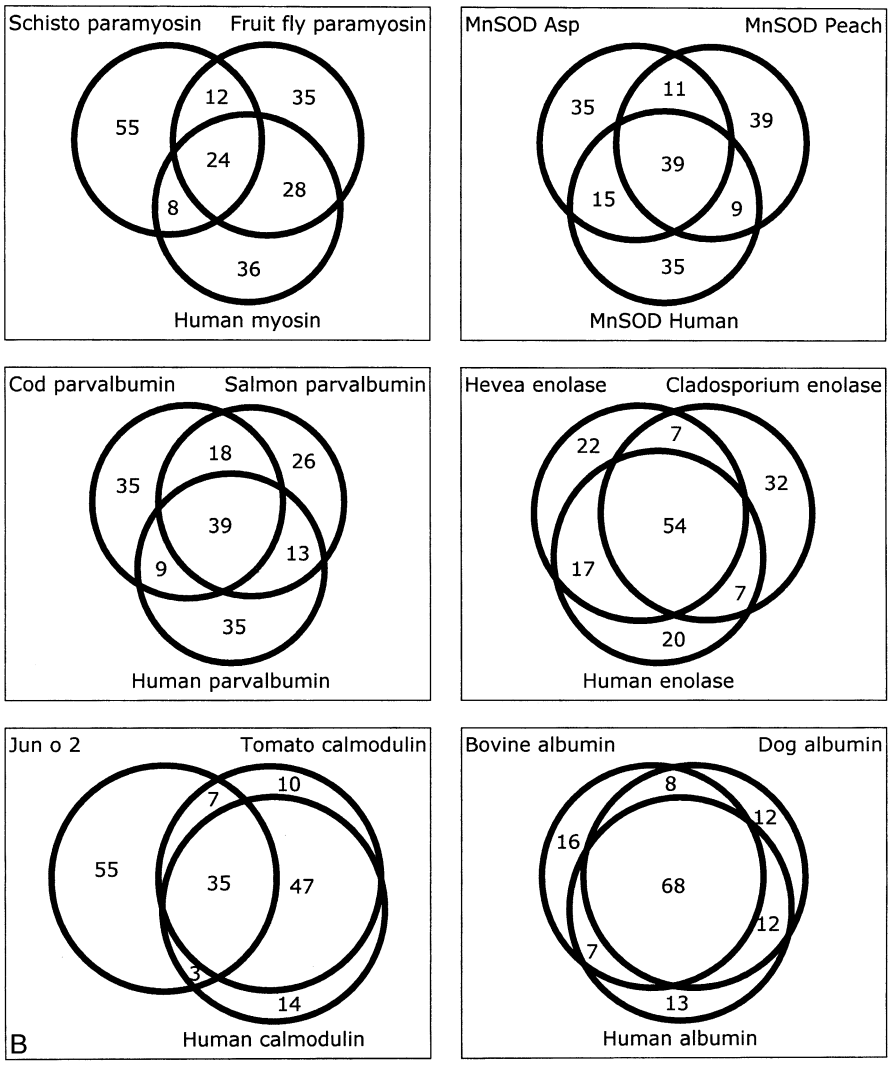

Figure 2B.

the preferential induction of cross-reacting antibodies, because the Th cells will, in principle, also stimulate B cells that are reactive with a non-cross-reactive epitope.

However, the situation is more complex in the case of B cells, because they contain a downregulatory $Fc_{gamma}RII$ receptor, which is relevant particularly in the case of naive B cells. On the one hand, just as for T cell priming, an antihapten immune response (induced by immunization with a hapten-carrier complex) is often enhanced by preimmunization with the same hapten coupled to a different carrier (90). On the other hand, the hapten (or cross-reacting epitope of the secondary antigen) will combine with the antibodies induced by the first immunization before binding to the B-cell receptor. If a naive B cell exists with a specificity for a novel (non-cross-reacting) epitope, this naive B cell will bind the immune complex. This will result in downregulation of naive B cells by the pre-existing antibodies. This effect, downregulation of an immune response by pre-existing antibodies, is the basis of the rhesus prophylaxis by administration of antirhesus antibodies.

Is the IgE response special in relation to cross-reactivity?

Cross-reactivity is certainly a prominent feature of the IgE response. Therefore, an obvious question is the following: is IgE more cross-reactive and, if so, why?

The first part of the question cannot convincingly be answered yet. There are some observations that seem to support this statement, but more data are certainly needed. Using the cat allergen Fel d 1 as a model, we found cross-reactivity with the ocelot equivalent to be more common for IgE than for IgG4 (IgE and IgG4 compared within the same serum): for IgE, the mean cross-reactivity was 66%, range 25–91%; for IgG4, it was 37%, range 14–60% ($P<0.001$) (91). Sera with IgE antibodies to *Dermatophagoides* group 1 or to grass-pollen group

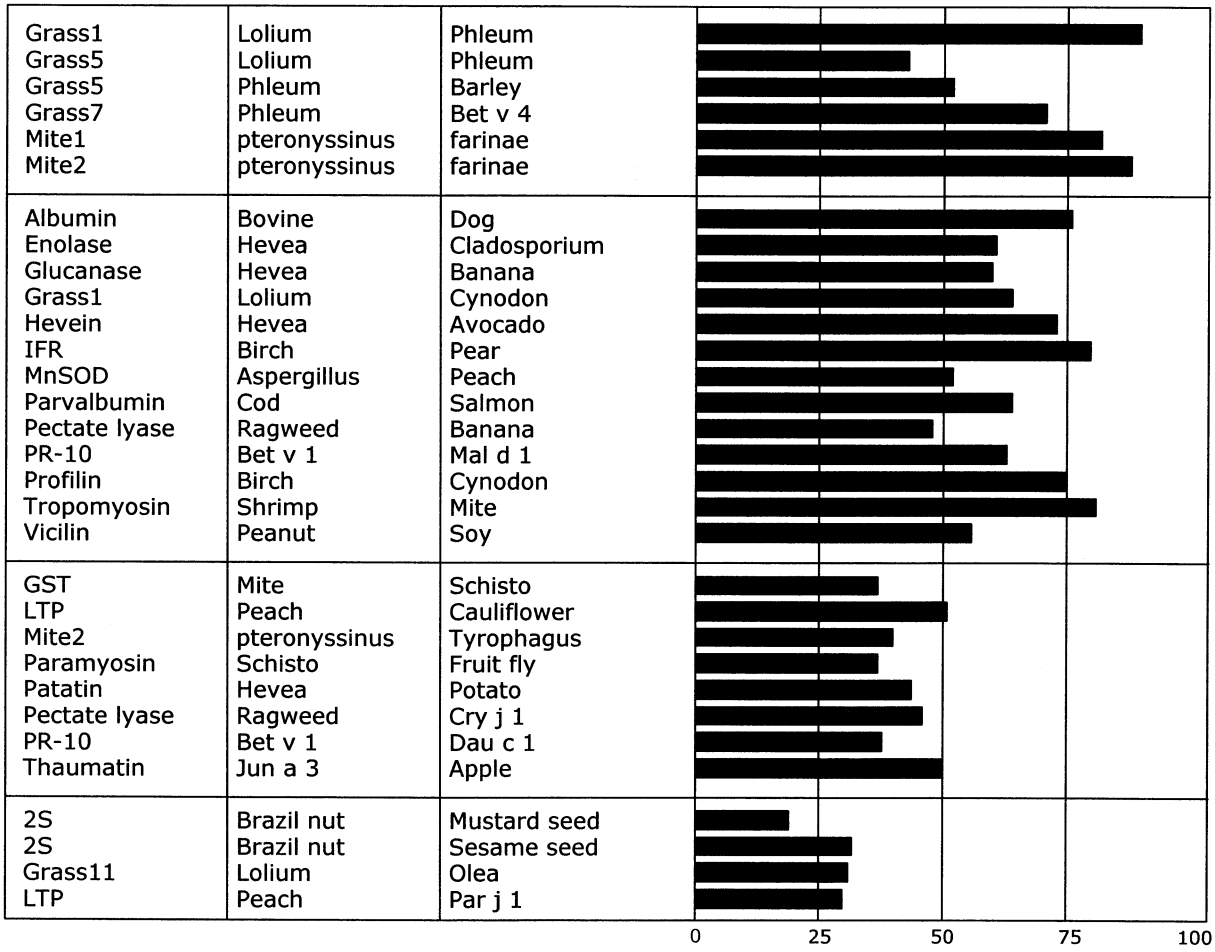

Figure 3. Amino-acid identity between pairs of proteins. Proteins have been grouped into "generally cross-reactive" (top panel), "often cross-reactive" (middle panel), and "rarely cross-reactive" (bottom panel).

1 allergens are usually cross-reactive with family members, whereas monoclonal antibodies are often found to be more specific.

If IgE is more cross-reactive (still a big "if"), why is it so? One possible answer is that this phenomenon is related to the number of antigenic contacts. In general terms, microbiologists tell us that early immune responses (disregarding the IgM response) tend to be more specific. In line with this, birch-apple cross-reactivity becomes more pronounced after long-standing pollinosis (92). This may be partially related to the phenomenon known as "the original antigenic sin" (93). This metaphor has been used to describe the lasting impression that the first influenza or HIV infection appears to have on the immune system. Upon subsequent infections with viral variants, the antibody response to the common epitopes is enhanced, whereas the response to new epitopes is suppressed, presumably due to the above-mentioned suppressive effects of pre-existing antibodies on naive B cells via $FcR_{gamma}II$. Therefore, the overall effect will be not only the expected stimulation of cross-reactive antibodies, but also suppression of non-cross-reactive antibodies. This effect, however, would explain only an increase in cross-reactivity via contacts with antigenic *variants*, whereas even repeated contact with the *same* antigen also seems to result in increased cross-reactivity. Presumably, this happens largely because of an increase in the polyclonality of the immune response, as is illustrated by the phenomenon called determinant spreading (85). The effect of polyclonality on overall cross-reactivity is a matter of statistics: the more diverse the antibody repertoire, the more likely it is to cause some cross-reactivity in an individual. If, for example, 20% of the antibodies to Bet v 1 are directed to cross-reactive epitopes and a birch-allergic patient has, on average, 10 B-cell clones activated by this allergen, the majority of patients will have a cross-reactive response, whereas most monoclonal antibodies would be non-cross-reactive. This increase in polyclonality following repeated exposure to the same antigen is a

clear indication that downregulation of naive B cells via $FcR_{gamma}II$ is not an absolute effect, since some B cells escape this downregulation. In a highly-selected model system, the human IgE response was found to be highly polyclonal (94).

In addition to the increase in polyclonality, repeated antigen contact also results in an increase in antibody affinity. Perhaps unexpectedly, this also might result in an increase in cross-reactivity. The relation between affinity and cross-reactivity is bimodal, with higher cross-reactivity among antibodies with a very low affinity as well as among those with a very high affinity, whereas antibodies with an intermediate reactivity are less cross-reactive. Low-affinity interactions often reflect a general tendency for interactions with a hydrophobic or highly charged surface patch. This type of interaction does not activate the immune regulatory circuits that result in tolerance induction. Thus, such low-affinity antibodies are often cross-reactive. As the affinity becomes higher, immune regulation becomes relevant, resulting in the downregulation of B-cell clones with reactivity to common structural motifs present in the autologous epitope repertoire. Cross-reactivity usually implies a lower affinity for the cross-reactive antigen compared to the primary antigen. Therefore, if the affinity to the primary antigen is already fairly low, the affinity for a related antigen will be even lower and thus presumably be insignificant. However, if the affinity for the primary antigen is very high, the affinity for a related antigen is much more likely to be significant.

If the formation of IgE antibodies requires repeated antigenic stimulation, the three mechanisms described above (original antigenic sin, polyclonality, and affinity maturation) may explain a relatively high prevalence of cross-reacting antibodies.

This is not an exclusive property of the IgE response. It is to be noted that the unexpected cross-reactivity pattern displayed by IgE antibodies to CCD is also not an exclusive property of IgE antibodies. Very similar cross-reactivity patterns have been described for rabbit IgG antibodies and human IgG4 antibodies (5, 95).

An interesting hypothesis has been put forward by Virtanen et al. (96) in relation to a discussion of whether proteins belonging to the lipocalin family are more likely to be allergenic. The proposal put forward was that the availability of human epitopes eliminates part of the potential immune repertoire and thus weakens the strength of the immune response. This, in turn, would favor Th2 over Th1 responses.

It is still an open question whether prior contact with an allergen via the airways increases or decreases the chance for cross-reactive responses after oral exposure to related antigens.

Can cross-reactivity be predicted?

It is tempting to try to extract rules for cross-reactivity from examples of homologous proteins with or without known IgE cross-reactivity (Fig. 3). Data on IgE cross-reactivity are, however, not always as clear-cut as one might wish. As mentioned before, complete absence of cross-reactivity cannot be proven, but theoretically a cross-reactivity scale could be made, ranging from "highly cross-reactive" (i.e., more than 50% of the IgE antibodies in a large serum pool are cross-reactive) to virtually not cross-reactive (i.e., less than 1% of the IgE antibodies are cross-reactive). For this classification to be useful, some relative affinity criterion needs to be included as well, preferably tested with soluble allergens. One approach is to use IgE binding to the cross-reactive allergen as the test system and compare the inhibition dose-response curves of the primary allergen with that of the cross-reactive allergen. If the dose required for 50% inhibition by the cross-reacting allergen were less than five times the dose required for the primary allergen, the cross-reactivity would be classified as high affinity. If the relative efficiency in this inhibition test were more than 25-fold lower, the cross-reactivity would be classified as low affinity. This is just an example of a possible approach, with a regrettable lack of scientific arguments for the factors 5 and 25. Some justification is found in the data presented for grass groups 1 and 5 (79). In this study, as in most other similar studies, no information was available on the absolute concentration of the cross-reacting allergens; therefore, differences in inhibitory efficiency could partially be explained by differences in allergen concentration.

This type of *quantitative* information is not available yet for most combinations of homologous allergens. Yet, *qualitative* information on cross-reactivity and lack of cross-reactivity is of interest as well. From the practical point of view, it is important to know whether cross-reactivity may be significant between proteins that belong to different protein-fold families. As discussed before, if two such proteins share a stretch of amino acids, this is unlikely to result in cross-reactivity unless these shared sequences both happen to be in unrestrained positions (C- or N-terminus, or perhaps a flexible loop). Within the field of allergy, no such cross-reactivity between proteins with different backbone folds has been found to result in relevant biologic effects.

Amino-acid homology is an imperfect substitute for epitope similarity. Epitopes are surface structures, whereas the most conserved part of the structure of proteins is the core. Moreover, mutations do not occur at random. Some features of a protein molecule are important for stability or function and are thus

less likely to be mutated. Yet, homology of less than 35% (less than one out of every three amino acids is identical) is unlikely to result in high-affinity cross-reactivity. High homology between two proteins is obviously more likely to result in cross-reactivity, but there is an additional factor to consider: the effect of tolerizing cross-reactivity.

The relevance of *tolerizing* cross-reactivity

The first contact with an antigen does not necessarily enhance the reaction following a subsequent contact. The most obvious factor shaping the immune repertoire is the spectrum of autologous epitopes. As a first approximation, autologous epitopes delete reactivity to epitopes on cross-reactive extraneous antigens. This tolerance is clearly not always complete, as immune reactivity to human proteins is well established (7, 97–99). Most likely, these autologous epitopes are (largely) hidden from the immune system. For the discussion of cross-reactivity patterns in general, the contribution of autologous epitopes is likely to be a strongly negative factor, i.e., a factor that limits the immunologic recognition of potentially cross-reactive structures in extraneous allergens. However, an interesting observation was made by Bernard et al. on the IgE response to β-casein (99). In 7/20 serum samples with high levels of IgE to this protein, some reactivity with the human homolog was found. This reactivity was inhibitable by the native bovine protein in solution, indicating that, in this case, the epitopes were not cryptic.

Another factor to consider is the role of tolerizing contact via the gastrointestinal tract. There is, however, not much information on food antigens suppressing sensitization to cross-reactive airborne allergens. Some potential examples exist, such as the effect of oral exposure to bovine serum albumin in milk in relation to cat (or dog) serum albumin.

Conclusions

Cross-reactivity has a clear structural basis: no relevant cross-reactivity without structural similarity. For globular proteins (i.e., for most allergens), structural similarity requires similarity in folding. Homology between two proteins that is limited to a small stretch of amino acids in the linear sequence is unlikely to result in significant cross-reactivity unless there is a similarity in the 3-D folding pattern.

Cross-reactivity means different things in different situations. Biologically, it makes a marked difference whether the cell involved is a T cell, B cell, or mast cell. More information is needed on the quantitative aspects: what is the relative avidity of the antibody toward the two antigens and what is the threshold avidity for biologic relevance of the cross-reactivity? This information is needed in order to translate immunochemically defined cross-reactivity into clinically relevant information.

Acknowledgments

We thank Erica van Oort, Ilona Kleine Budde, and Steven Stapel for allowing the use of their experimental results and for valuable discussions.

References

1. Spitzauer S. Allergy to mammalian proteins: at the borderline between foreign and self? Int Arch Allergy Immunol 1999;**120**:259–269.
2. van Ree R, Driessen MNBM, van Leeuwen WA, Stapel SO, Aalberse RC. Variability of crossreactivity of IgE antibodies to group I and V allergens in eight grass pollen species. Clin Exp Allergy 1992;**22**:611–617.
3. Anderson LBJ, Dreyfuss EM, Logan J, Johnstone DE, Glaser J. Melon and banana sensitivity coincident with ragweed pollinosis. J Allergy 1970;**45**: 310–319.
4. Hannuksela M, Lahti A. Immediate reactions to fruits and vegetables. Contact Dermatitis 1977;**3**:79–84.
5. Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. J Allergy Clin Immunol 1981;**68**:356–364.
6. Valenta R, Duchene M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergens. J Exp Med 1992;**175**:377–385.
7. Valenta R, Duchene M, Pettenburger K, et al. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. Science 1991; **253**:557–560.
8. Kremser M, Lindemayr W. Celery allergy (celery contact urticaria syndrome) and relation to allergies to other plant antigens. Wien Klin Wochenschr 1983;**95**:838–843.
9. M'Raihi L, Charpin D, Pons A, Bongrand P, Vervloet D. Cross-reactivity between latex and banana. J Allergy Clin Immunol 1991;**87**:129–130.
10. De Maat B, Akkerdaas JH, van Ree R, Aalberse RC. Vineyard snail allergy possibly induced by sensitization to house-dust mite (*Dermatophagoides pteronyssinus*). Allergy 1995;**50**: 438–440.
11. van Ree R, Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F. Possible induction of food allergy during mite immunotherapy. Allergy 1996;**51**:108–113.
12. van Ree R, Antonicelli L, Akkerdaas JH, et al. Asthma after consumption of snails in house-dust-mite-allergic patients: a case of IgE cross-reactivity. Allergy 1996;**51**:387–393.

13. Witteman AM, Akkerdaas JH, van Leeuwen J, van der Zee JS, Aalberse RC. Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects. Int Arch Allergy Immunol 1994;**105**:56–61.
14. Witteman AM, van den Oudenrijn S, van Leeuwen J, Akkerdaas J, van der Zee JS, Aalberse RC. IgE antibodies reactive with silverfish, cockroach and chironomid are frequently found in mite-positive allergic patients. Int Arch Allergy Immunol 1995;**108**:165–169.
15. Björkstén F, Halmepuro L, Hannuksela M, Lahti A. Extraction and properties of apple allergens. Allergy 1980;**35**:671–677.
16. Prahl P, Weeke B, Løwenstein H. Quantitative immunoelectrophoretic analysis of extract from cow hair and dander. Characterization of the antigens and identification of the allergens. Allergy 1978;**33**:241–253.
17. Andersen KE, Løwenstein H. An investigation of the possible immunological relationship between allergen extracts from birch pollen, hazelnut, potato and apple. Contact Dermatitis 1978;**4**:73–79.
18. Calkhoven PG, Aalbers M, Koshte VL, Pos O, Oei HD, Aalberse RC. Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. Allergy 1987;**42**:382–390.
19. Hsieh LS, Moos M, Lin Y. Characterization of apple 18 and 31 kd allergens by microsequencing and evaluation of their content during storage and ripening. J Allergy Clin Immunol 1995; **96**:960–970.
20. Inschlag C, Hoffmann-Sommergruber K, O'Riordain G, et al. Biochemical characterization of Pru a 2, a 23-kD thaumatin-like protein representing a potential major allergen in cherry (*Prunus avium*). Int Arch Allergy Immunol 1998;**116**:22–28.
21. Karamloo F, Schmitz N, Scheurer S, et al. Molecular cloning and characterization of a birch pollen minor allergen, Bet v 5, belonging to a family of isoflavone reductase-related proteins. J Allergy Clin Immunol 1999;**104**: 991–999.
22. Pauli G, Bessot JC, Dietemann-Molard A, Braun PA, Thierry R. Celery sensitivity: clinical and immunological correlations with pollen allergy. Clin Allergy 1985;**15**:273–279.
23. Dietschi R, Wüthrich B, Johansson SGO. Zum sogenannten "Sellerie-Karotten-Beifuss-Gewuerz-Syndrom". RAST-Resultate mit neuen Gewuerzdiscs. Z Hautkr 1987;**62**: 524–531.
24. Luttkopf D, Ballmer-Weber BK, Wüthrich B, Vieths S. Celery allergens in patients with positive double-blind placebo-controlled food challenge. J Allergy Clin Immunol 2000;**106**: 390–399.
25. Stager J, Wüthrich B, Johansson SGO. Spice allergy in celery-sensitive patients. Allergy 1991;**46**:475–478.
26. Vieths S, Jankiewicz A, Wüthrich B, Baltes W. Immunoblot study of IgE binding allergens in celery roots. Ann Allergy Asthma Immunol 1995;**75**: 48–55.
27. Wüthrich B, Stager J, Johansson SGO. Celery allergy associated with birch and mugwort pollinosis. Allergy 1990;**45**: 566–571.
28. Ballmer-Weber BK, Vieths S, Luttkopf D, Heuschmann P, Wüthrich B. Celery allergy confirmed by double-blind, placebo-controlled food challenge: a clinical study in 32 subjects with a history of adverse reactions to celery root. J Allergy Clin Immunol 2000;**106**:373–378.
29. Vallier P, Dechamp C, Vial O, Deviller P. A study of allergens in celery with cross-sensitivity to mugwort and birch pollens. Clin Allergy 1988; **18**:491–500.
30. Vallier P, Dechamp C, Valenta R, Vial O, Deviller P. Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species. Identification as a profilin. Clin Exp Allergy 1992;**22**:774–782.
31. Jankiewicz A, Aulepp H, Baltes W, et al. Allergic sensitization to naive and heated celery root in pollen- sensitive patients investigated by skin test and IgE binding. Int Arch Allergy Immunol 1996;**111**:268–278.
32. Heiss S, Fischer S, Hirschwehr R, et al. A 60 kD trypsin resistant component of mugwort pollen represents a novel cross-reactive structure in pollen, fruit and vegetable allergy [Abstract]. International Congress Immunology, 1995:205–205.
33. Ganglberger E, Radauer C, Grimm R, et al. N-Terminal sequences of high molecular weight allergens from celery tuber. Clin Exp Allergy 2000;**30**: 566–570.
34. Bauer L, Ebner C, Hirschwehr R, et al. IgE cross-reactivity between birch pollen, mugwort pollen and celery is due to at least three distinct cross-reacting allergens: immunoblot investigation of the birch-mugwort-celery syndrome. Clin Exp Allergy 1996;**26**:1161–1170.
35. Diaz-Perales A, Lombardero M, Sanchez-Monge R, et al. Lipid-transfer proteins as potential plant panallergens: cross-reactivity among proteins of *Artemisia* pollen, *Castanea* nut and Rosaceae fruits, with different IgE-binding capacities. Clin Exp Allergy 2000;**30**:1403–1410.
36. de la Hoz F, Polo F, Moscoso DP, Selles JG, Lombardero M, Carreira J. Purification of *Art v* I, a relevant allergen of *Artemisia vulgaris* pollen. Mol Immunol 1990;**27**:651–657.
37. Breiteneder H, Ebner C. Molecular and biochemical classification of plant-derived food allergens. J Allergy Clin Immunol 2000;**106**:(1 Pt 1) 27–36.
38. Sanchez-Monge R, Lombardero M, Garcia-Selles FJ, Barber D, Salcedo G. Lipid-transfer proteins are relevant allergens in fruit allergy. J Allergy Clin Immunol 1999;**103**:514–519.
39. Pastorello EA, Farioli L, Pravettoni V, et al. The major allergen of peach (*Prunus persica*) is a lipid transfer protein. J Allergy Clin Immunol 1999; **103**:520–526.
40. Asero R, Mistrello G, Roncarolo D, et al. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. Int Arch Allergy Immunol 2000;**122**:20–32.
41. Blanco C, Carrillo T, Castillo R, Quiralte J, Cuevas M. Latex allergy: clinical features and cross-reactivity with fruits. Ann Allergy 1994;**73**: 309–314.
42. Kurup VP, Fink JN. The spectrum of immunologic sensitization in latex allergy. Allergy 2001;**56**:2–12.
43. Alenius H, Kalkkinen N, Lukka M, et al. Prohevein from the rubber tree (*Hevea brasiliensis*) is a major latex allergen. Clin Exp Allergy 1995;**25**: 659–665.
44. Sowka S, Hsieh LS, Krebitz M, et al. Identification and cloning of Prs a 1, a 32-kDa endochitinase and major allergen of avocado, and its expression in the yeast *Pichia pastoris*. J Biol Chem 1998;**273**:28091–28097.
45. Alenius H, Kalkkinen N, Reunala T, Turjanmaa K, Palosuo T. The main IgE-binding epitope of a major latex allergen, prohevein, is present in its N-terminal 43-amino acid fragment, hevein. J Immunol 1996;**156**:1618–1625.
46. Beezhold DH, Sussman GL, Liss GM, Chang NS. Latex allergy can induce clinical reactions to specific foods. Clin Exp Allergy 1996;**26**:416–422.
47. Breiteneder H, Sowka S, Wagner S, et al. Cloning of the patatin-like latex allergen Hev b 7, its expression in the yeast *Pichia pastoris* and its immunological characterization. Int Arch Allergy Immunol 1999;**118**:309–310.

48. Sowka S, Wagner S, Krebitz M, et al. cDNA cloning of the 43-kDa latex allergen Hev b 7 with sequence similarity to patatins and its expression in the yeast *Pichia pastoris*. Eur J Biochem 1998;**255**:213–219.
49. Seppälä U, Alenius H, Turjanmaa K, Reunala T, Palosuo T, Kalkkinen N. Identification of patatin as a novel allergen for children with positive skin prick test responses to raw potato. J Allergy Clin Immunol 1999;**103**: 165–171.
50. Sowka S, Hafner C, Radauer C, et al. Molecular and immunologic characterization of new isoforms of the *Hevea brasiliensis* latex allergen Hev b 7: evidence of no cross-reactivity between Hev b 7 isoforms and potato patatin and proteins from avocado and banana. J Allergy Clin Immunol 1999;**104**: 1302–1310.
51. Koshte VL, Kagen SL, Aalberse RC. Crossreactivity of IgE antibodies to caddis fly with Arthropoda and Mollusca. J Allergy Clin Immunol 1989;**84**:174–183.
52. Tsai LC, Chao PL, Hung MW, et al. Protein sequence analysis and mapping of IgE and IgG epitopes of an allergenic 98-kDa *Dermatophagoides farinae* paramyosin, Der f 11. Allergy 2000; **55**:141–147.
53. van Kuik JA, van Halbeek H, Kamerling JP, Vliegenthart JFG. Primary structure of the low-molecular weight carbohydrate chains of *Helix pomatia* α-hemocyanin. J Biol Chem 1985;**260**:13984–13988.
54. van Kuik A, Sijbesma RP, Kamerling JP, Vliegenthart JFG, Wood EJ. Primary structure of a low-molecular-mass N-linked oligosaccharide from hemocyanin of *Lymnaea stagnalis*. 3-*O*-methyl-D-mannose as a constituent of the xylose-containing core structure in an animal glycoprotein. Eur J Biochem 1986;**160**:621–625.
55. van Kuik JA, Hoffman RA, Mutsaers JHGM, van Halbeek H, Kamerling JP, Vliegenthart JFG. A 500 MHz 1H-NMR study on the N-linked carbohydrate chain of bromelain. Glycoconj J 1986;**3**:27–34.
56. D'Andrea G, Bouwstra JB, Kamerling JP, Vliegenthart JFG. Primary structure of the xylose-containing N-linked carbohydrate moiety from ascorbic acid oxydase of *Cucurbita pepo medullosa*. Glycoconj J 1988;**5**:151–157.
57. Faye L, Gomord V, Fitchette-Lainé AC, Chrispeels MJ. Affinity purification of antibodies specific for Asn-linked glycans containing α1–3 fucose or β1–2 xylose. Anal Biochem 1993;**209**:104–108.
58. Hayashi M, Tsuru A, Mitsui T, et al. Structure and biosynthesis of the xylose-containing carbohydrate moiety of rice alpha-amylase. Eur J Biochem 1990;**191**:287–295.
59. Faye L, Johnson KD, Sturm A, Chrispeels MJ. Structure, biosynthesis, and function of asparagine-linked glycans on plant glycoproteins. Physiologia Plantarum 1989;**75**: 309–314.
60. Ishihara H, Takahashi N, Oguri S, Tejima S. Complete structure of the carbohydrate moiety of stem bromelain. An application of the almond glycopeptidase for structural studies of glycopeptides. J Biol Chem 1979; **254**:10715–10719.
61. Kubelka V, Altmann F, Staudacher E, et al. Primary structure of the N-linked carbohydrate chains from honeybee venom phospholipase A_2. Eur J Biochem 1993;**213**:1193–1204.
62. Laurière M, Laurière C, Chrispeels MJ, Johnson K, Sturm A. Characterization of a complex xylose-specific antiserum that reacts with the complex asparagine-linked glycans of extracellular and vacuolar glycoproteins. Plant Physiol 1989;**90**:1182–1188.
63. Yasuda Y, Takahashi N, Murachi T. The composition and structure of carbohydrate moiety of stem bromelain. Biochemistry 1970;**9**:25–32.
64. Lalles JP, Peltre G. Biochemical features of grain legume allergens in humans and animals. Nutr Rev 1996; **55**:101–107.
65. Staudacher E, Altmann F, März L, Hard K, Kamerling JP, Vliegenthart JFG. α1–6(α1–3)-difucosylation of the asparagine-bound *N*-acetylglucosamine in honeybee venom phospholipase A_2. Glycoconj J 1992;**9**:82–85.
66. Takahashi N, Hotta T, Ishihara H, et al. Xylose-containing common structural unit in N-linked oligosaccharides of laccase from sycamore cells. Biochemistry 1986;**25**:388–395.
67. Tretter V, Altmann F, Kubelka V, März L, Becker WM. Fucose α 1,3-linked to the core region of glycoprotein *N*-glycans creates an important epitope for IgE from honeybee venom allergic individuals. Int Arch Allergy Immunol 1993; **102**:259–266.
68. Fotisch K, Altmann F, Haustein D, Vieths S. Involvement of carbohydrate epitopes in the IgE response of celery-allergic patients. Int Arch Allergy Immunol 1999;**120**:30–42.
69. Ueda H, Ogawa H. Glycobiology of the plant glycoprotein epitope: structure, immunogenicity and allergenicity of plant glycotopes. Trends Glycosci Glycotechnol 1999;**11**:413–428.
70. van Ree R, Cabanes-Macheteau M, Akkerdaas J, et al. Beta(1, 2)-xylose and alpha(1, 3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. J Biol Chem 2000; **275**:11451–11458.
71. Elgavish S, Shaanan B. Structures of the *Erythrina corallodendron* lectin and of its complexes with mono- and disaccharides. J Mol Biol 1998; **277**:917–932.
72. Shaanan B, Lis H, Sharon N. Structure of a legume lectin with an ordered N-linked carbohydrate in complex with lactose. Science 1991;**254**:862–866.
73. van der Veen MJ, van Ree R, Aalberse RC, et al. Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. J Allergy Clin Immunol 1997;**100**: 327–334.
74. Batanero E, Crespo JF, Monsalve RI, Martin-Esteban M, Villalba M, Rodriguez R. IgE-binding and histamine-release capabilities of the main carbohydrate component isolated from the major allergen of olive tree pollen, Ole e 1. J Allergy Clin Immunol 1999;**103**:147–153.
75. Ohta M, Shigeta S, Ono K, Takao T, Shimonishi Y, Oka S. Sugar sequences of allergenically active oligosaccharide alcohols isolated from a large-molecular-size sea squirt antigen termed H-antigen. Arch Biochem Biophys 1989;**275**:151–165.
76. Ohta M, Matsuura F, Kobayashi Y, Shigeta S, Ono K, Oka S. Further characterization of allergenically active oligosaccharitols isolated from a sea squirt H-antigen. Arch Biochem Biophys 1991;**290**:474–483.
77. Shigeta S, Ono K, Ohta M, Kobayashi Y, Oka S. *N*-Acetylgalactosaminyl disaccharide terminals contained in presumed carbohydrate epitopes specific to sea-squirt allergy. J Biochem (Tokyo) 1989;**105**:691–696.
78. Shigeta S, Okamura M, Tsutsumi M, et al. Structure of an allergenic pentasaccharitol, Gp-1β-b6, isolated from a sea squirt antigen, Gi-rep, as a minimum structural unit responsible for its allergenicity. J Biochem (Tokyo) 1990;**108**:47–52.
79. van Ree R, van Leeuwen WA, Aalberse RC. How far can we simplify *in vitro* diagnostics for grass pollen allergy?: a study with 17 whole pollen extracts and purified natural and recombinant major allergens. J Allergy Clin Immunol 1998; **102**:184–190.

80. van Ree R, van Leeuwen WA, Akkerdaas JH, Aalberse RC. How far can we simplify *in vitro* diagnostics for Fagales tree pollen allergy? A study with three whole pollen extracts and purified natural and recombinant allergens. Clin Exp Allergy 1999;**29**:848–855.
81. Asero R. Effects of birch pollen-specific immunotherapy on apple allergy in birch pollen-hypersensitive patients. Clin Exp Allergy 1998;**28**:1368–1373.
82. Fernandez-Rivas M, van Ree R, Cuevas M. Allergy to Rosaceae fruits without related pollinosis. J Allergy Clin Immunol 1997;**100**:728–733.
83. van Ree R, Fernandez-Rivas M, Cuevas M, van Wijngaarden M, Aalberse RC. Pollen-related allergy to peach and apple: an important role for profilin. J.Allergy Clin Immunol 1995;**95**: 726–734.
84. Aalberse RC. The IgE response and atopy. Eur Respir J 1991;**4**:78s–84s.
85. Mohapatra SS. Determinant spreading: implications in allergic disorders. Immunol Today 1994;**15**:596–597.
86. Aalberse RC. Allergen: the trigger. Allergy Clin Immunol Int 2000 **(Suppl)**:13–15.
87. van Ree R, Akkerdaas JH, van Leeuwen A, et al. New perspectives for the diagnosis of food allergy. Allergy Clin Immunol Int 2000;**12**:7–12.
88. van Ree R. Factors modulating allergen-induced histamine release. In: Bousquet J, Yssel H, editors. Immunotherapy in asthma. New York: Marcel Dekker, 1999:399–409.
89. Paul WE, Katz DH, Goidl EA, Benacerraf B. Carrier function in anti-hapten immune responses. II. Specific properties of carrier cells capable of enhancing anti-hapten responses. J Exp Med 1970;**132**:283–299.
90. Koch G, Osmond DG, Julius MH, Benner R. The mechanism of thymus-dependent antibody formation in bone marrow. J Immunol 1981;**126**: 1447–1451.
91. van Milligen FJ, van Swieten P, Aalberse RC. IgE and IgG4 binding to the ocelot variant of the cat (*Felis domesticus*) major allergen Fel d 1. Allergy 1994;**49**:393–394.
92. Asero R, Massironi F, Velati C. Detection of prognostic factors for oral allergy syndrome in patients with birch pollen hypersensitivity. J Allergy Clin Immunol 1996;**97**:611–616.
93. Fazekas de St groth B, Webster RG. Disquisitions on original antigenic sin. I. Evidence in man. J Exp Med 1966; **140**:2893–2898.
94. van't Hof W, van den Berg M, Driedijk PC, Aalberse RC. Heterogeneity in the IgE binding to a peptide derived from the house dust mite allergen *Der p* II indicates that the IgE response is highly polyclonal. Int Arch Allergy Immunol 1993;**101**:437–441.
95. van Ree R, Aalberse RC. Demonstration of carbohydrate-specific IgG4 antibodies in sera of patients receiving grass pollen immunotherapy. Int Arch Allergy Immunol 1995;**106**: 146–148.
96. Virtanen T, Zeiler T, Rautiainen J, Mantyjarvi R. Allergy to lipocalins: a consequence of misguided T-cell recognition of self and nonself? Immunol Today 1999;**20**:398–400.
97. Valenta R, Maurer D, Steiner R, et al. Immunoglobulin E response to human proteins in atopic patients. J Invest Dermatol 1996;**107**:203–208.
98. Mayer C, Appenzeller U, Seelbach H, et al. Humoral and cell-mediated autoimmune reactions to human acidic ribosomal P2 protein in individuals sensitized to *Aspergillus fumigatus* P2 protein. J Exp Med 1999;**189**: 1507–1512.
99. Bernard H, Negroni L, Chatel JM, et al. Molecular basis of IgE cross-reactivity between human beta-casein and bovine beta-casein, a major allergen of milk. Mol Immunol 2000;**37**:161–167.