

Allergic Crossreactions

General and Practical Aspects

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Allergic Crossreactions: Observations and Mechanisms

Evidence of crossreactions between different allergens has been increasingly reported for the last 15 yr (1–10). Although the first clinical observations dealt with crossreactions between food allergens and inhaled allergens, the concept of allergic crossreaction is now extended to a more “immunological” meaning, i.e., recognition of, and reaction to, a common epitope irrespective of the route of contact with the allergen.

In fact, “crossreaction” is an umbrella term for several different clinical situations. Table 1 gives some examples of typical observations and their immunological interpretation:

1. The allergen is “hidden” and, in this case, the allergenic molecular identity is complete (1);
2. The allergen is common to taxonomically closely related species and the allergenic molecular identity is partial (2–5); and
3. An epitope is common to taxonomically nonrelated species, or even to different phyla, and nevertheless is at the origin of clinical symptoms (6–10).

Cases 1 and 2 are clinically relevant, and the identification of a common allergen within two (or more) different sources is important for prevention. The immunological basis, however, is rather simple: the same molecule induces the immune response and is responsible for the allergic symptoms, whatever its source or route of contact. In case 3,

Table 1
Observations of Crossreactions Between Allergens,
and Their Immunological Significance

Clinical observations	Immunological significance
Asthma from inhaled garlic dust with asthma from garlic-containing food (1)	"Hidden" allergen, complete allergenic identity
Asthma from inhaled bird allergens with allergic skin reactions or digestive symptoms after eating eggs (2-4)	Common allergen, partial allergenic identity, same or closely related species
Food allergy to shrimp with food allergy to crab or lobster (5)	Common allergen, partial allergenic identity, different but closely related species
Food allergy to shrimp with food allergy to mollusks (6)	Common allergen, partial allergenic identity, different and sometimes nonrelated species or families
Respiratory allergy to pollens or latex with food allergy to fruit and/or vegetables (7,8)	
Respiratory allergy to house dust mite with food allergy to snails or crustacea (9,10)	Common epitope, different allergenic molecules, different phyla

usually the substances in question share only one epitope. It can remain unrecognized until revealed by some clinical evidence, and the particularities of the individual immune response can be responsible for different consequences ranging from the absence of sensitization to overt clinical symptoms.

Demonstrating Crossreactivity

Demonstration of immunological crossreactions in IgE-dependent immune responses relies on the inhibition of allergen-IgE binding by the crossreacting allergen. Incubation of the anti-A and anti-B IgE-containing serum from a patient allergic to allergen A, with a potentially crossreacting allergen B, prevents secondary binding of serum IgE to allergen A. This binding inhibition can be measured by any immunoassay adapted to IgE measurement: RAST, ELISA, immunoblotting... Comparative immunoblottings of allergenic fractions of allergen A and B can give the molecular weight and/or isoelectric points of potentially crossreacting fractions, and can identify their numbers and frequencies in a given population of patients, showing similar IgE-binding patterns. This has been shown, for instance, for taxonomically diverse pollens (11). Only specific inhibition of IgE-binding to a given band of allergen B extract by prior incubation of allergen A- and B-containing serum, with allergen A, and vice-versa, however, can prove the existence of

the crossreactivity. Subsequent investigations using purified fractions and recombinant allergens, as well as monoclonal antibodies (MAbs), are necessary to first identify the crossreacting allergen and, then, the specific crossreacting epitope. This strategy has been used to demonstrate the existence and nature of the crossreacting allergens involved in pollen/fruit and vegetables (12–16), crustacea/mollusks (17), latex/fruit and vegetables (18,19), shrimp/cockroach (20), or *Dermatophagoides pteronyssinus*/snail (10,21,22), or crustacea (23) crossreactions. However, it must be stressed that in vitro demonstration of an immunological crossreactivity is not synonymous with sensitization and/or clinical manifestations of allergy, and this has major consequences for the clinical management of allergic patients.

Why Allergic Crossreactivity?

The specificity of the immune response is governed by the specific recognition of antigens by T- and B-cell receptors. The diversity of both T-cell (TCR) and B-cell (BCR, or surface immunoglobulins) receptors comes from a genetic rearrangement that takes place at the beginning of cell differentiation (24). This gives rise to hundreds of millions of different cell clones; every clone is characterized by the “paratope” of its specific receptor, i.e., the hypervariable sequence of amino acids in the variable domain of the polypeptide chains of either TCR (V α and V β) or BCR/surface Ig (VL and VH). As already hypothesized at the end of the 19th century by Paul Erlich, and only demonstrated at the end of this century, the B- and T-cell repertoires are anticipatory; i.e., appropriate receptors exist prior to encountering antigens. Every human being is thus characterized by his or her own repertoire: a personal set of specific clones that defines the extent and types of complementary structures (the “epitopes”) that can be specifically recognized and, after specific binding, can lead to an effector immune response. Allergens are environmental foreign antigens that are able to induce specific immune responses, typically through IgE-dependent mechanisms or through delayed-type-hypersensitivity (DTH) reactions. In both cases, T cells are required because they are necessary for a B-cell switch to IgE production (25) and for initiating cell recruitment and cytotoxicity in DTH (26). T cells can recognize only antigen fragments, the T-cell epitopes, after an adequate processing by antigen-presenting cells. T-cell epitopes, usually related to the linear structure of the antigenic molecule, 7–25 amino acids long, are presented to the TCR in association with molecules encoded by the major histocompatibility complex, the HLA-class I and II molecules. HLA-class II molecules are particularly involved in allergen recognition, since they are specialized in the presentation of exogenous, foreign antigens (27). Theoretically,

all molecules that can be processed to end up with the same epitope can induce the activation of the same T-cell clone irrespective of their source, spatial conformation, or function.

B-cell epitopes can be recognized by the BCR on intact antigens; their size is usually larger than that of T-cell epitopes; and they can be associated with the tertiary or quaternary structure of the antigenic molecule (conformational epitopes), and with the polysaccharide or lipid moiety of the molecules. As the variable portion of the secreted Ig, whatever its isotype, is identical to the binding site of the BCR on the same clone, the same binding properties apply both to the receptor (the BCR) and to the effector molecule (the Ig). It is thus conceivable that, after prior sensitization of B cells by a given molecule from a given source (either inhaled or ingested), secondary binding of the same epitope, present on a different molecule from a different source (and via another route), can provoke mast-cell degranulation through IgE bridging on its surface.

Why Is Allergic Crossreactivity Unpredictable?

If (theoretically) any identical epitopes, hidden in different molecules, can lead to crossreactivity, then epidemiological observations and *in vitro* immunological studies (using immunoelectrophoresis or immunoblotting) clearly show that crossreactions are not clinically expressed in all allergic individuals (7,11,17,20,28,30). Four different situations can be encountered:

1. Absence of any demonstrable crossreactivity (even if crossreaction were theoretically possible);
2. Crossreaction demonstrated *in vitro* by the presence of specific IgE to two or more different extracts or molecules, reciprocally inhibited by prior incubation;
3. *In vivo* sensitization demonstrated by concomitant positivity of prick/intradermal tests or patch-tests; and
4. Clinical manifestations of allergy after contact with the cross-reacting allergens, irrespective of the route of administration.

The absence of any *in vitro* demonstrable crossreaction in a part of an allergic population, despite the existence of a proven crossreactivity between two allergens, reflects the forementioned personalized characteristics of the immune response. Every so-called "allergen" is in fact a mixture of allergenic components, as can be seen clearly in the immunoblotting profile (11,31). They themselves are composed of single epitopes. The definition of "major" and "minor" allergenic components relies on the frequency of their recognition by serum IgE from a population of allergic patients. This definition may also apply to single epitopes. Among all possible epitopes from a given source, only

those corresponding to effectively rearranged lymphocyte receptors (the individual repertoire) can induce an immune response (24); moreover, the particularities of antigen presentation to T cells (which involves genetically highly polymorphic HLA molecules) give additional restrictions owing to the possible inadequacy of the presentation and, thus, to the failure to activate T cells (27). It has been demonstrated that there are a variety of T-cell epitopes involved in *D. pteronyssinus* allergy (32), and this may account for the unpredictability of cross-reactivities with related or unrelated mites (33,34), and completely unrelated species, like snails (9,10,22).

Moreover, individual differences in isotype production (IgG vs IgE for instance), in the affinity/avidity of the secreted IgE, can also be involved in the presence/absence of demonstrable crossreactivity. Besides qualitative aspects, specific IgE levels seem to play a role in the appearance of a double sensitization: skin and RAST reactivity to oysters occurs in crustacea-sensitive subjects with high levels of anticrustacea antibodies. Factors such as exercise, hormonal changes, concomitant infectious diseases, and environmental pollution; even stressful events may be necessary to transform a latent sensitization into an allergic disease (37). Even if (theoretically) minute amounts of allergens can induce anaphylactic shock, the size of the allergen load can also play a role in the appearance of symptoms, especially after ingestion (37). It has been suggested that the allergenic load administered via a parenteral route, created by allergen immunotherapy, could be a potential risk factor for revealing latent cross sensitization (9,21). This could be owing to the repeated antigenic stimulation leading to the recruitment of new B-cell clones and/or hypermutations and affinity maturation.

Why Do Allergens Crossreact?

During the last 10 yr, molecular biology research has yielded some insight into the enigma of the allergenic property of particular antigens and, thus, into the puzzling question of crossreactivity (37,38). Complementary DNAs, coding for a number of allergens, were characterized and expressed as recombinant allergens in heterologous expression systems; MAbs have also been used to search for epitope identities in molecules from different sources (12,21,23,31,32,38,40). It appears that a majority of allergens are represented by "conserved proteins" (i.e., proteins fulfilling important biological functions) and are therefore conserved in their sequence and/or structure. This includes a variety of enzymes, as found in mites (32,39,41), hymenoptera (42), or pollen allergens (37,38,43); pathogenesis-related proteins (44); proteins associated with the cytoskeleton or muscle system, like profilins (15,16,18,37,38)

or tropomyosin (10,23,31); calcium-binding proteins (45); or hemocyanin (22). Using an antiserum raised against β -fructosidase isolated from suspension-cultured carrot cells, crossreactivity has been demonstrated with a number of proteins present in many seed plants (Gymnosperms as well as Monocotyledons and Dicotyledons) and in invertebrates, crustacea, mollusks, and insects, including honeybee phospholipase A and *Helix pomatia* hemocyanin (46). The shared epitope consisted of a small xylose-containing asparagine-linked glycan, not present in mammals but identified in plants and invertebrate glycoproteins, that could increase the immunogenicity of the proteins to which they are attached (46). This study on IgG crossreactivity suggests that nonpolypeptide epitopes can also play a role in allergic crossreactivity.

Practically Speaking

Recognition of allergic crossreactions is of major importance for the management of allergic patients. What were once incomprehensible clinical associations or syndromes can now be explained by the demonstration of the shared epitope. Use of recombinant allergens, such as rBet v1 and rBet v2 (birch profilin), may help the epidemiologist to delineate the crossreacting profiles of allergic patients in a given population. On an individual basis, knowledge of the immunological significance of crossreactivity should help the clinician to interpret the results of *in vivo* and *in vitro* testing accurately, and to give sound advice to allergic patients. Demonstration of IgE binding to a crossreacting allergen in the serum is not synonymous with allergy; even if the patient needs to be aware of this possibility, it is not advisable to eliminate all crossreacting foods, for instance, in the absence of any prior clinical manifestations. Any results of specific IgE measurement and skin tests must be interpreted with reference to crossreactions; on the other hand, the history of the allergic patient must also be interpreted with this reference, and sound questions should be asked to uncover the associated clinical manifestations when crossreactivities are possible.

In fact, it is now well-established that primary sensitization may result from contact with an environmental allergen (e.g., airborne pollen allergens, German cockroach, or house dust mites) leading to the induction of IgE antibodies that are then capable of crossreacting with another environmental allergen containing the shared epitope (e.g., plant-derived food [38], shrimps [20], or snails [9,10], respectively). Presence of pollen-reactive IgE antibodies may not necessarily be a true reflection of sensitizing pollen species (11); presence of specific IgE antibodies to snails has been demonstrated in children allergic to *D. pteronyssinus* who had never eaten snails or other mollusks (48).

Conversely, it is conceivable that persistent contact with a crossreacting allergen could be responsible for the persistence of symptoms, despite avoidance of the sensitizing and so-called offending allergen, as has been the case even in occupational allergies (49). Specific immunotherapy using allergenic extracts has been reported to suppress clinical manifestations of allergy to the crossreacting allergen (50) and to favor and reveal the clinical expression of allergy to the crossreacting allergen (9,10,21,51). Further studies are clearly needed to assess the relevance of these observations and clarify the actual benefit/risk of hyposensitization.

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