

Basic Features of Crossreactivity in Tree and Grass Pollen Allergy

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Introduction

The term "crossreactivity" is a somewhat redundant concept that is often applied to both antigens and antibodies. In the following pages, crossreaction is used solely to describe the situation where an antibody, here IgE, raised against an epitope on an antigen is able to react with an identical or similar epitope on a different antigen. Antibodies may crossreact; antigens do not.

Given the above definition, there are a number of theoretical ways in which an allergic individual's symptomatic crossreactivity toward various allergen sources can manifest itself:

1. IgE antibodies reacting with common epitopes;
2. IgE antibodies raised against species-specific epitopes originating from a number of sensitizations involving different allergen sources;
3. IgE antibodies raised against highly homologous proteins commonly found in different allergen source materials; and
4. Any combination of 1-3.

Patients exhibiting sensitivity toward more than one allergen source may therefore be either monosensitized (IgE antibodies raised against allergen[s] from only one source) or multisensitized (IgE antibodies raised against allergens from two or more sources). On the symptomatic level, the patients all seem to crossreact, because the commonly used diagnostic measures (clinical history, skin prick test, specific IgE measurements, and so forth) are unable to differentiate such sensitization patterns.

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The allergic individual's macroscopic or symptomatic manifestations of crossreactivity raise a number of fundamental questions concerning diagnosis and, in particular, immunotherapy of allergic individuals. With regard to in vitro and in vivo diagnostic procedures, it is most probably less important which particular species are utilized in the test procedure; e.g., *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* can be substituted freely in diagnosing house dust mite allergy (1,2), and the same species independence can probably be applied to tree and grass pollen diagnostic tests. However, the step from diagnosis to immunotherapy involves the selection of the appropriate therapeutic agent (i.e., the allergen extract[s] matching the individual patients' sensitization source). Is an exact match optimal? Or is an appropriate treatment one that includes one or more of the allergen sources toward which the patient's IgE antibodies crossreact? The questions are closely linked to the immunological mechanism(s) behind successful immunotherapy, and, currently, no clinical or immunological data are available to answer these questions.

The allergen manufacturer and the physicians are faced with the dilemma of transferring the results of clinical history and diagnostic tests into a recommendation of an appropriate treatment schedule. The matter is further complicated by geographical and climatic differences that strongly influence the distribution, number, and diversity of the sensitization sources in the environment of the individual patients. Furthermore, individual allergens are not homogeneous, since they all seem to exist in multiple isoforms that diverge from one another by a few amino acid substitutions, multiplying the epitope spectrum considerably. The allergen manufacturer should aim at producing extracts that contain all possible allergens, as well as the most common isoforms of the individual allergens in a given extract, in order to mimic the naturally occurring epitope spectrum. The following sections describe in more detail the problems involved in the characterization of the antibody responses toward the common allergen sources of tree and grass pollens.

Tree Pollen Allergens

Pollens of the *Fagales* order are the most important allergen source during spring in the temperate climate zone of the Northern hemisphere (3). Patients allergic to birch pollen often, if not always, react toward allergens from pollens of the taxonomically related species (alder, hazel, hornbeam, and so forth) (4–6). A substantial fraction of these patients exhibit additional sensitivity toward certain fruits and vegetables (7–12).

Detailed investigations of the specificity of the patients' IgE antibodies indicate that the majority of patients are sensitized by allergens derived from birch pollen, primarily Bet v 1, but all patients express IgE antibodies crossreacting with allergens from the botanically related species (6,13).

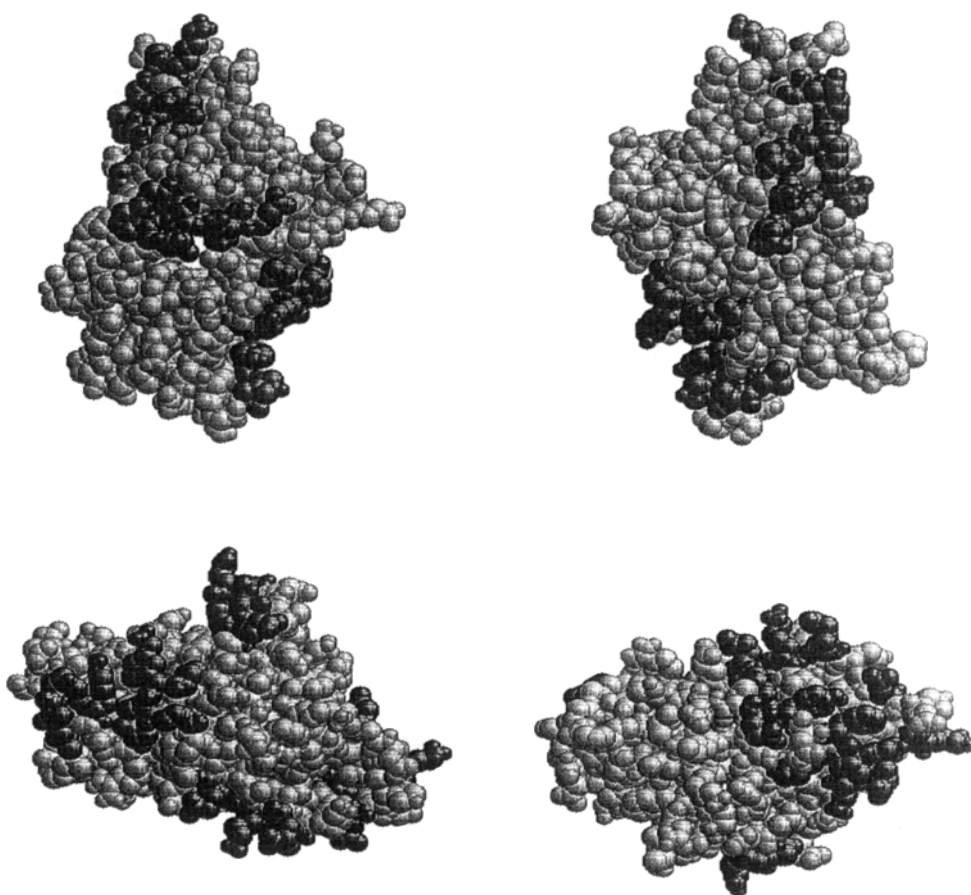


Fig. 1. Space-filling models of Bet v 1.2801, viewed from various angles. The dark gray colored residues are surface-exposed amino acids that are strictly conserved in 56 known complete amino acid sequences of *Fagales* major allergens homologous to Bet v 1.

Recently, the three-dimensional (3D) structure of the major allergen of birch pollen, Bet v 1, was determined (14,15) and combined with a large body of primary sequence data for Bet v 1 isoforms and homologous allergens from related species; a model of the epitope pattern that drives the antibody crossreactivity may evolve. Figure 1 shows the space-filling models of Bet v 1 viewed from different angles. The dark gray colored residues are strictly conserved in 56 *Fagales* group 1 sequences. The invariant residues cluster into three surface-exposed patches, each covering surface areas greater than 600 Å²; thus, each patch is adequately large enough to harbor antibody-binding epitopes. If an allergic individual raises IgE antibodies reactive with one of more of these patches, the patient will experience sensitivity toward the

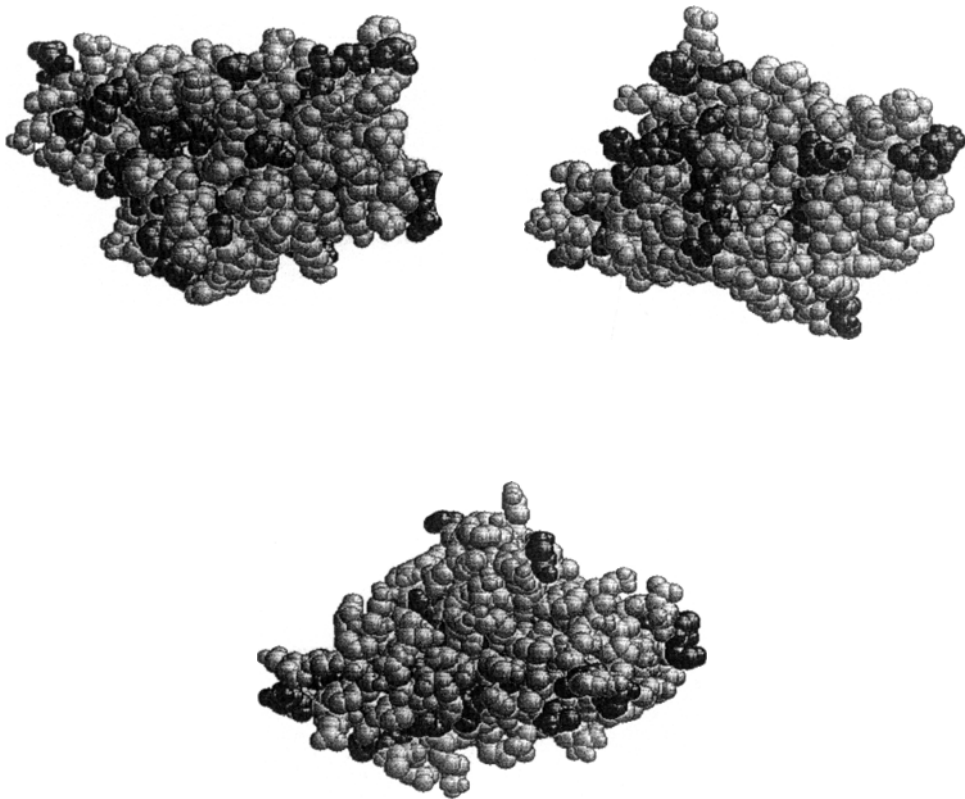


Fig. 2. Space-filling models of Bet v 1.2801, viewed from various angles. The dark gray colored residues are those that differ in the sequences between Bet v 1.2801 and Car b 1.0102.

majority, if not all, pollens originating from the *Fagales* order. Figure 2 illustrates the comparison of two species. The residues, which differ between Bet v 1.2801 and Car b 1.0102, are colored dark gray, and it is obvious that large surface areas are identical on the two allergens, indicating that the existence of antibodies crossreactive with group 1 allergens from the two species are highly probable. Therefore, *Fagales* group 1 allergen epitopes may be divided into groups according to occurrence:

1. Universally common epitopes;
2. Epitopes common between a limited number of species; and
3. Strictly species-specific epitopes.

The results obtained from the structural analysis substantiates the conclusions drawn from the analysis of an individual patient's cross-reactivity pattern, namely that crossreacting IgE antibodies are the rule rather than the exception (6). Additionally, *Fagales* pollen extracts contain profilin, which is identified in both fruits and vegetables, as well as

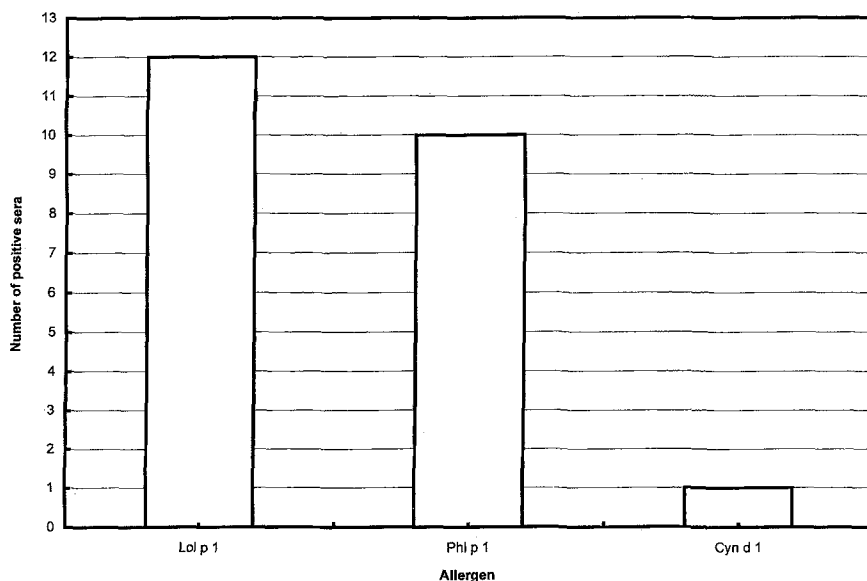


Fig. 3. Grass group 1 allergogram. The columns represent the number of patients' sera reactive with *Phl p 1*, *Lol p 1*, and *Cyn d 1* in SDS-PAGE IgE immunoblots.

pollens from completely different species, i.e., grass pollens (16–19). Reactivity toward profilin may account in part for the IgE cross-reactivity observed in a patient.

Clinical investigations performed with different Scandinavian patient populations clearly indicate that immunotherapy with birch pollen extract alone is as efficient as immunotherapy performed with a mixture of pollen extracts of different *Fagales* species, probably reflecting the primary sensitization role of birch pollen in the Scandinavian area.

Grass Pollen Allergens

The grasses constitute a large and diverse group of plants that include more than 10,000 individual species growing throughout the world. Some of the species inhabit only a few climate zones, whereas others are found more or less worldwide. The allergen and antigen composition of the individual grass extracts are complicated, and several important allergens can be identified for each species. The group 1 and possibly the group 2/3 allergens are present in the pollens of all the grasses investigated so far, whereas the group 5 allergens seem to be present only in the pollens of the "temperate" species (*Phleum p.*, *Lolium p.*, *Poa p.*, and so on), not in the "subtropical" species (*Cynodon d.*, *Paspalum n.*, *Sorghum h.*, and so on) (20). Figure 3 illustrates the number of sera in a Scandinavian sample population ($n = 13$) of grass pollen

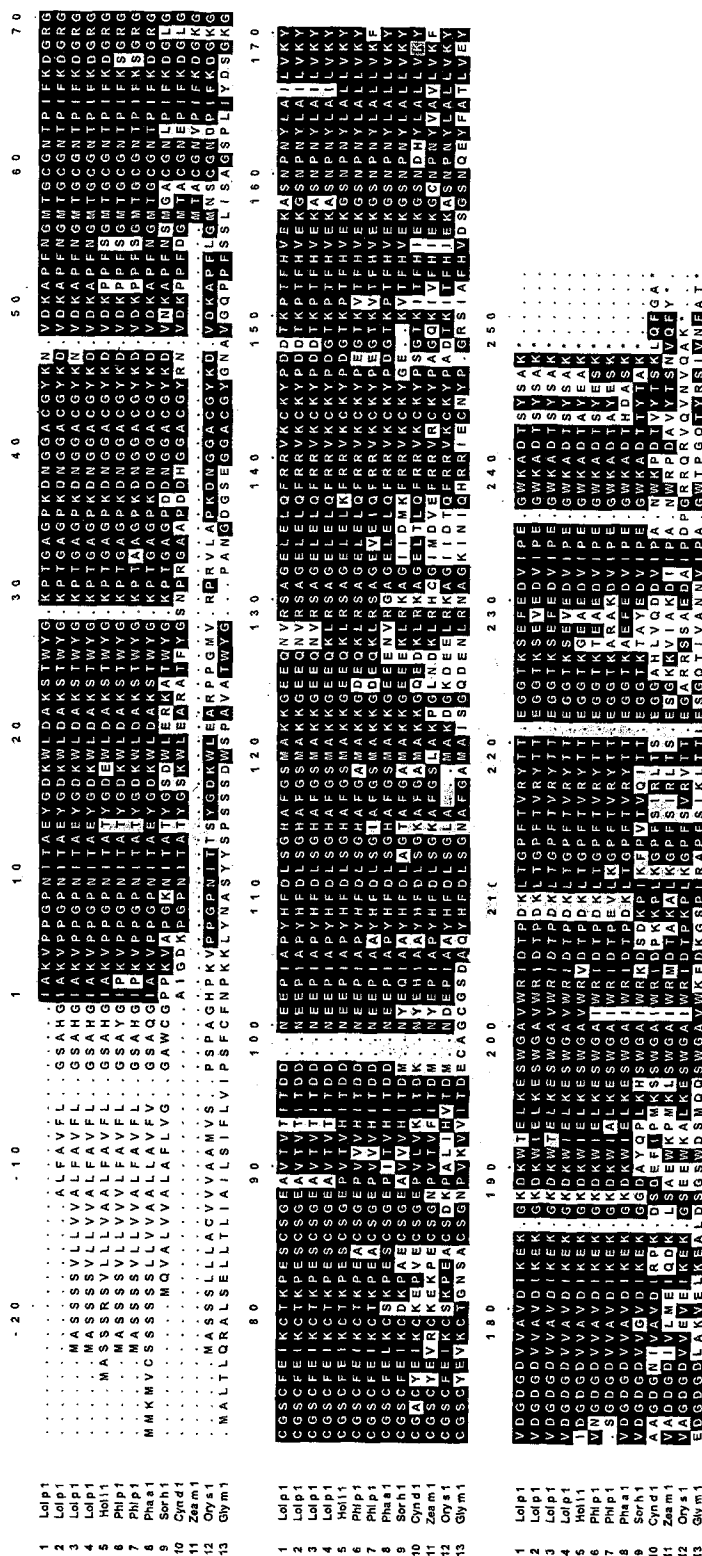


Fig. 4. Comparison of grass group 1 amino acid sequences. The identical amino acids are indicated by a gray background and gaps by dots.

allergic individuals recognizing the group 1 allergens of *Phleum p.*, *Lolium p.*, and *Cynodon d.* Only a few of the patients' sera contained IgE crossreactive with the group 1 allergen from *Cynodon d.*, whereas the patients' serum IgE reacted equally well with the group 1 allergens from the temperate grasses. The IgE crossreactivity pattern, combined with experiments performed with murine monoclonal antibodies raised against different group 1 allergens (not shown), strongly indicate that the epitope structure of the group 1 allergens may differ considerably, even though the primary amino acid sequences data are highly homologous (Fig. 4). The difference between the Circular Dichroism spectra of "temperate" and "subtropical" group 1 allergens (not shown) indicates that the allergens differ at least with respect to secondary structural elements and probably also in their overall 3D structure. The apparent structural differences between "temperate" and "subtropical" grass group 1 allergens and the discrepancy of the content of group 5 allergens strongly suggest that both types of species should be included in test panels, and eventually in treatment regimen, if the patients live or have been sensitized in geographical areas where both "temperate" and "subtropical" grass species coexist.

Concluding Remarks

Optimal immunotherapy should be directed toward the allergen(s) to which an individual is actually sensitized. However, in many patients crossreactions and multiallergenic manifestations involving obvious and/or obscure allergens highlight the problem of our currently inadequate diagnostic techniques. Some patients may be receiving suboptimal treatment because it is difficult to identify the sensitizing allergen source(s) without employing elaborate and costly measures. Some crucial questions—such as (1) What is the immunological mechanism involved in successful immunotherapy? and (2) Should the treatment allergen extract match the patients' sensitization source?—need to be answered in order to provide both the diagnostic and treatment measures that may be optimal for the majority of allergic individuals.

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