

Immunobiology of Grass Pollen Allergens

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Among pollen allergens, grass pollen allergens are some of the most frequent contributors to allergic symptoms. Substantial progress has been made since the 1960s in the identification and characterization of the grass allergens. Members of this group belong to the Poaceae family, and have been classified into 13 distinct groups based on their structure, and their biological and immunologic properties. The major contributors to allergy and, hence, most studied among the grass allergens, are those belonging to groups 1 and 5. This review is focused on the structure and immunobiology of the grass allergens and highlights how recent advances in the field have contributed to superior diagnosis and immunotherapy for allergy to grass pollens.

Introduction

Pollen from the grass family Poaceae is one of the major causes of allergy worldwide during spring and summer months, commonly known as “allergy season,” affecting approximately 40% of allergic individuals [1,2•,3,4]. Pollen can trigger allergic rhinitis and asthma symptoms in some patients. Attempts to study these allergens began around the 1960s, and since then more than 130 allergens have been identified. Recent advances in immunology and biochemistry have allowed extensive characterization and purification of allergenic molecules as well as the synthesis of recombinant allergens [1].

Classification and Nomenclature

Grasses belong to the Poaceae family, which is the fourth largest group of flowering plants. They are classified into five subfamilies based on the system of Watson and Dallwitz [5]. Almost all allergenic species belong to Pooideae, Chloridoideae, or Panicoideae subfamilies. Pooideae can be found mainly in the temperate zones,

such as the Mediterranean area. They represent 70% to 85% of the grasses in the northwestern United States and Canada, approximately 40% to 50% in the middle states, and 15% to 25% in the southern states. This subfamily includes species such as smooth brome, orchard, Kentucky blue, canary, velvet, Timothy, and ryegrass. Chloridoideae are widespread throughout North America, Africa, and Australia. Members of this family include grama grass, salt grass, and Bermuda grass. They represent more than 50% of the grasses in the southwestern United States and less than 10% in the northwestern United States. Panicoideae are mainly found in the more humid and tropical environments of the savannas of Indonesia and Africa, and northeastern South America. This family includes Bahia grass and Johnson grass and accounts for more than 75% of the grasses in the Panama Canal Zone, 50% in the southern United States, and only 5% in the northwestern United States.

The current accepted nomenclature for allergens is outlined by the World Health Organization (WHO) and the International Union of Immunological Societies (IUAC) [6]. An allergen identified by SDS-PAGE and isoelectric point is named by the first three letters of the genus and the first letter of the species. A number is assigned to different allergens from the same species. For example, an allergen from *Lolium perenne* (ryegrass) is called Lol p 1. A list of the groups of allergens that have been characterized to date are shown in Table 1. Approximately 14 species, with a list of different allergens known thus far, are listed in Table 2. This list is updated on a regular basis and can be found in the Allergome database [7]. An allergen is considered to be major if the purified form of the allergen induces an immediate skin test response in more than 50% of the allergic population. It is a minor allergen if the immediate skin test response occurs in less than 50% of allergic individuals [6].

Characteristics and Immunobiology

Attempts to characterize the allergenic proteins of grass pollen can be traced back to the late 19th century when Charles Blackley identified pollen as the cause of hay fever [8,9]. However, studies conducted by King and Norman [10] in the early 1960s pioneered purification and characterization studies of allergens. Hybridoma and protein purification technologies allowed the characterization of

Table 1. Classification of grass pollen allergens according IUIS nomenclature system

Allergen	Source	Characterisitcs	Molecular weight, kDa	Isoelectric point	Patient reactivity, %
Group 1	Perennial ryegrass, timothy, velvet, Johnson, Bermuda grass	Acidic glycoproteins, β -expansins	27–35	N/A	95
Group 2	Perennial ryegrass, orchard grass	Acidic protein	11	5, 5.1–5.3	60
Group 3	Perennial ryegrass, orchard grass	Basic protein	11–14	9–9.4	60
Group 4	Timothy, orchard, and Bermuda grass	Basic glycoprotein	50–60	8.6–10.4	70
Group 5 (9)	Panicoid, chloridoid, arundinoid grass, and velvet grass pollen	Heterogeneous, ribonucleases	27–38	4.2–7.0	65–85
Group 6	Timothy grass	Polypeptides	12–13	9–10.2	60–70
Group 7	Bermuda, timothy grass	Ca ²⁺ -binding proteins	8–12	5.2–5.5	10
Group 10	Timothy, perennial ryegrass, Bermuda grass	Cytochrome C	11	N/A	10
Group 11	Timothy, maize, tomato, olive	Glycoprotein, trypsin inhibitors	16–18	10	> 65
Group 12	Timothy, Bermuda, birch pollen	Profilin, actin-binding protein	13–14	5–6	20
Group 13	Timothy grass pollen	Polygalacturonase	50–60	N/A	50

Adapted from Esch [14•] and Weber [27]. IUIS—International Union of Immunological Societies.

many allergens in the 1970s. Monoclonal antibodies are produced to a diverse range of allergens that allow sufficient purification for structural analysis [11,12]. The usefulness of this method is limited, as it does not allow for therapeutic studies, and the antigenic epitopes that the mouse monoclonal IgG antibodies recognize may differ from the allergenic epitopes recognized by human IgE molecules. The emergence of superior molecular biology techniques, such as gene cloning, gene expression, DNA sequencing, polymerase chain reaction (PCR) genomics, and proteomics have facilitated the characterization of more grass pollen allergens. This had lead to the identification of the primary structures of these molecules [13].

The allergenicity of the molecule is attributed to the proteins that are released from the pollen grain once it is hydrated. To date, there are 13 groups of grass pollen allergens that have been identified and characterized [14•]. Many of the allergens have been shown to exist in isoforms. These isoforms can be differentiated from each other based on their size or primary structure.

Structure

Most allergens are mainly proteins, and their folded structure is important to the allergenicity of the molecule. It is this folded structure, along with other properties that induces IgE production in allergic individuals. Although allergens as a group have no characteristic structural features, there are highly conserved regions among family members. These regions are responsible for the homology and cross-reactivity of these allergens. Epitopes on the surface of the folded proteins are what engage immune cells and lead to sensitization upon initial exposure to

allergen. Epitopes are those sequences on the protein surface that interact with the Fab fragment of the antibody and set off the cascade of events leading to an allergic response. Contrary to previous theories, it is now thought that all protein epitopes are conformational [15], a finding that has been validated by the x-ray crystallography studies of Fischmann *et al.* [16] in 1991. With the recent advances in the proteomics, there are numerous investigations into the three-dimensional structure of allergens, and the findings seem to corroborate the conformational theory. To date, only the structures of some members of the Timothy grass family have been solved and published in the NCBI Entrez database under “3D domains” [17].

Complete allergens have the ability to sensitize, bind IgE molecules, and elicit an allergic reaction. However, incomplete allergens can elicit allergic symptoms but cannot sensitize the individual. Due to similar structural motifs among some allergens, some incomplete allergens can bind to IgE molecules already present and specific to a related allergen, and induce an allergic reaction [15]. The structural biology of allergens is important in predicting the allergic potential of newly discovered grass allergens and in predicting the cross-reactivity of these molecules. It can also be useful in allergy therapeutics, in that if the structure can predict the function of the allergen and possible cross-reactive species, preventive measures can be taken.

Cross-reactivity

Many grass species may have structural similarities, owing to conserved immunodominant residues that facilitate cross-reactivity among the groups. Elucidation of primary structures and B-cell and T-cell epitopes has lead to the

Table 2. A list of grass pollen allergens characterized to date

Common name	Species name	IUIS molecules and biological functions
Redtop grass	<i>Agrostis alba</i>	Agr a 1 (expansins)
Crested wheat grass (quack grass)	<i>Agropyron cristatum</i>	Agr c CBP (calcium-binding proteins)
Sweet vernal grass	<i>Anthoxanthum odoratum</i>	Ant o 1 (expansins); Ant o 12 (actin-binding proteins); Ant o 13 (polygalacturonases); Ant o 2; Ant o 4; Ant o 5 (ribonucleases); Ant o 6; Ant o CBP (calcium-binding proteins)
Cultivated oat grass	<i>Avena sativa</i>	Ave s 1 (expansins); Ave s 12 (actin-binding proteins); Ave s 13 (polygalacturonases); Ave s 2; Ave s 4; Ave s 5; Ave s CBP (calcium-binding proteins)
Blue grama grass	<i>Bouteloua gracilis</i>	Bou g 1
Field brome grass	<i>Bromus arvensis</i>	Bro a 4
Smooth brome grass	<i>Bromus inermis</i>	Bro i CBP (calcium-binding proteins)
Bermuda grass	<i>Cynodon dactylon</i>	Cyn d 1; Cyn d 10; Cyn d 12 (actin-binding proteins); Cyn d 13 (polygalacturonases); Cyn d 15; Cyn d 2; Cyn d 22 (glycolytic enzymes); Cyn d 23; Cyn d 24 (PR-protein); Cyn d 4; Cyn d 5 (ribonucleases); Cyn d 6; Cyn d 7 (calcium-binding proteins); Cyn d BG-60 (flavoproteins)
Cocksfoot grass (orchard grass)	<i>Dactylis glomerata</i>	Dac g 1 (expansins); Dac g 13 (polygalacturonases); Dac g 2 (unknown); Dac g 3; Dac g 4; Dac g 5 (ribonucleases); Dac g CBP (calcium-binding proteins); Dac g Profilin (actin-binding proteins)
Salt grass	<i>Distichlis spicata</i>	Dis s 1; Dis CBP (calcium-binding proteins)
Reed fescue grass	<i>Festuca elatior</i>	Fes e 1; Fes e 13; Fes e 4; Fes e 5; Fes e CBP (calcium-binding proteins)
Meadow fescue	<i>Festuca pratensis</i>	Fes p 1; Fes p 13; Fes p 4; Fes p 5
	<i>Festuca rubra</i>	Fes r 1; Fes r 5
Velvet grass	<i>Holcus lanatus</i>	Hol l 1 (expansins); Hol l 2; Hol l 4; Hol l 5 (ribonucleases)
Barley	<i>Hordeum vulgare</i>	Hor v 1; Hor v 12; Hor v 13; Hor v 2; Hor v 4; Hor v 5
Cogon grass	<i>Imperata cylindrica</i>	Imp c 4; Imp c 5 (ribonucleases); Imp c VIIIe1
Italian ryegrass	<i>Lolium multiflorum</i>	Lol m 5
Rye grass	<i>Lolium perenne</i>	Lol p 1 (expansins); Lol p 10 (cytochrome C); Lol p 11 (trypsin inhibitors); Lol p 12 (actin-binding proteins); Lol p 13 (polygalacturonases); Lol p 2 (unknown); Lol p 3; Lol p 4; Lol p 5 (ribonucleases); Lol p CBP (calcium-binding proteins)
Rice	<i>Oryza sativa</i>	Ory s 1; Ory s 12; Ory s 2
Bahia grass	<i>Paspalum notatum</i>	Pas n 1
Canary grass	<i>Phalaris aquatica</i>	Pha a 1 (expansins); Pha a 5 (ribonucleases)
Timothy grass	<i>Phleum pratense</i>	Phl p 1 (expansins); Phl p 11 (trypsin inhibitors); Phl p 12 (actin-binding proteins); Phl p 13 (polygalacturonases); Phl p 2 (unknown); Phl p 3; Phl p 4 (pectate lysates); Phl p 5 (ribonucleases); Phl p 6; Phl p 7 (calcium-binding proteins); Phl p P1-P2-P5-P6; Phl p P2-P6; Phl p P5-P1; Phl p P6-P2
Common reed	<i>Phragmites australis</i>	Phr a 1; Phr a 12; Phr a 13; Phr a 4; Phr a 5
June grass (Kentucky blue grass)	<i>Poa pratensis</i>	Poa p 1 (expansins); Poa p 10 (cytochrome C); Poa p 12 (actin-binding proteins); Poa p 13 (polygalacturonases); Poa p 2; Poa p 4; Poa p 5 (ribonucleases); Poa p 6; Poa p CBP (calcium-binding proteins)
Rye	<i>Secale cereale</i>	Sec c 1 (a); Sec c 12; Sec c 13; Sec c 2; Sec c 4; Sec c 5
Johnson grass	<i>Sorghum halepense</i>	Sor h 1; Sor h CBP (calcium-binding proteins); Sor h Profilin (actin-binding proteins)
Wheat	<i>Triticum aestivum</i>	Tri a 1; Tri a 12; Tri a 13; Tri a 2; Tri a 3; Tri a 4; Tri a 5; Tri a CBP (calcium-binding proteins)
Maize	<i>Zea mays</i>	Zea m 1; Zea m 12; Zea m 13; Zea m 4; Zea m 5; Zea m CBP; Zea m Zml3

Adapted from the Allergome database <http://www.allergome.org>. IUIS—International Union of Immunological Societies.

identification of cross-reactive species [18,19]. The cross-reactivity of allergens is determined by its structure. Cross-reactivity occurs not only among the grasses but with other aeroallergens from other sources [20,21]. The scope of this review is limited to cross-reactivity among the grasses. Many proteins undergo post-translational modifications in the endoplasmic reticulum during processing. A common modification to grass allergens is glycosylation of asparagine, serine, and threonine residues. The glycosylated residues can act as epitopes and, therefore, can also be a source of cross-reactivity for grass allergens [15]. Although the fold of the protein is critical to the cross-reactivity between allergens, the primary structure plays a role, also. It holds true that cross-reacting molecules share similar conformational folds, but all proteins with the same folds are not necessarily cross-reacting.

Numerous studies have been carried out to investigate grass pollen allergen cross-reactivity. These studies use radioallergosorbent tests (RAST), enzyme-linked immunosorbent assay (ELISA) inhibition, and crossed radioimmunoelectrophoresis (CRIE) to reveal the cross-reactive species [22–29]. Cross-reactivity is thought to be based on the relatedness of the allergens. The more closely related the source of the allergen, the more likely the cross-reactivity, which is possibly due to the plant evolution from a common progenitor [30,31]. On this premise, plants in the same genus should have the largest number of shared epitopes. Studies have shown this theory to be correct. Most of the major allergens belong to three main subfamilies: Chloridoideae, Pooideae, and Panicoideae. Using a human serum pool from allergic North Americans with extracts from each of the three groups, Martin *et al.* [31] showed that although Chloridoid pollen and Poid pollen cross-react with Panicoid pollen, they do not cross-react with each other. Other groups have conducted similar studies to confirm these findings [32,33]. Recently, Davies *et al.* [27] have shown that patients who are allergic to ryegrass showed limited cross-reactivity to Bahia grass pollen extract. This indicates that Bahia grass should be considered when diagnosing and treating hay fever in the late summer months when Bahia grass flowers are in bloom.

Many grass species are present in the same geographic location, and, therefore, an individual can be sensitized simultaneously to pollens from many different species. Therefore, incomplete allergens can stimulate an immune response by cross-reacting with immunoglobulin E (IgE) molecules generated to similar allergens. To date, most grass pollen extracts have not been tested for cross-reactivity, and the data remain fairly limited [30] (Fig. 1).

B-cell and T-cell Epitopes

Thirteen allergen groups have been identified to date (Table 2). This review covers grass pollen allergens from all these groups. The most significant allergens resulting in

human allergies belong to groups 1 and 5, and, therefore, these are the allergens that are extensively studied for their B-cell and T-cell epitopes. B-cell epitopes are recognized by IgE molecules and are thought to be divided into two classes: continuous and discontinuous epitopes. Continuous epitopes are short, linear stretches of primary structure that can be recognized, whereas discontinuous epitopes are amino acid sequences that can only be recognized when the molecule is folded. Because proteins assume a natural three-dimensional structure, the latter theory is more widely accepted. IgE production and eosinophilia are dependent on the activation and subsequent cytokine secretion from allergen-specific CD4⁺ T lymphocytes. Type 1 allergic reactions are usually a Th2 type response, and the interleukin (IL)-4 produced stimulates B cells to switch to IgE production. IL-5 is secreted and helps in the maturation and differentiation of eosinophils [34]. The identification of epitopes on the allergen molecules can lead to the development of novel immunotherapy strategies.

Group 1 allergens are probably the most important group of allergens as they are among the most important mediators of type 1 allergic reactions worldwide. Most studies employ recombinant technology to identify epitopes on the allergens. The Timothy grass pollen allergen Phl p1 can be used as the model allergen for group 1, because it contains most of the B-cell as well as the T-cell epitopes displayed by group 1 allergens [35]. The IgE-reactive fragments are highly conserved among group 1 allergens and account for the high degree of cross-reactivity among this group. Group 5 allergen Hol l 5, a major allergen of velvet grass pollen, has also been studied for its epitopes [36]. Recombinant studies revealed four different continuous IgE epitopes as well as five discontinuous epitopes on the Hol l 5 fragments. These epitopes are distributed over the entire molecule and are individually recognized by IgE antibodies for different patients [36]. Hol l 5, therefore, can be thought of as the model group 5 antigen. IgE-binding sites have also been identified on other group 5 allergens, such as Phl p 5a [37,38] and Lol p 5 [36].

New Developments

Diagnosis

Allergen microarrays or allergen chips are an interesting tool that can be used to diagnose allergy. In recent years, there have been tremendous advancements in the field of biochip technology. This has led to the development of DNA microarrays and, more recently, protein arrays. This technique is advantageous, because it uses small amounts of sample and can be used for diagnosis. Jahn-Schmid *et al.* [39•] have studied the analytic performance of an allergen microarray for the detection of allergen-specific IgE from patient serum. They found that, when compared with the CAP/RAST system, the microarray was more sensitive with respect to the antigens used, and the specificity was about

affect the recognition or the activation of the basophils. This indicates that complexing allergen with nanoparticles does not alter their allergenicity and, therefore, allows further study into the use of NPs as a novel delivery method. In terms of immunotherapy, studies are currently being conducted to investigate the safety of an allergen tablet for specific immunotherapy [43•]. Another study is looking at grass pollen injection immunotherapy [44•]. This group has shown that, with this therapy, there is a suppression of allergen-induced, late-phase skin responses.

It was demonstrated that a few point mutations in the major ryegrass allergen Lol p 5 sequence could produce mutants with reduced allergenic activity [45]. Recently, Metz-Favre *et al.* [41•] showed that recombinant molecules expressing a spectrum of grass pollen epitopes can be engineered. They fused four major allergens of Timothy grass pollen and found they contained most of the relevant B-cell and T-cell epitopes that are present in natural grass pollens. If the allergen fragments are reshuffled, the overall allergenic activity will be reduced. Therefore, it appears possible to engineer hybrid molecules with hypoallergenic derivatives. When used for vaccination, these molecules might be able to induce strong protective IgG antibody responses.

Conclusions

Progress in structural, biological, and immunologic characterization of more than 100 grass pollen allergens have led to significant advances in our understanding of not only the intrinsic molecular properties of these molecules but also the immune response-generating characteristics. Also, the studies to date of these allergens have opened new avenues for diagnosis of grass pollen allergy and the potential to improve immunotherapy with mutated hypoallergenic molecules.

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