

7 Grass Pollen Allergens

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INTRODUCTION

Grass pollens represent a major component of the airborne allergen load during the spring and summer months in most parts of the world. They are responsible for the symptoms in the majority of allergic rhinitis patients and can also trigger asthma. The diagnosis and treatment of grass pollen allergy with grass pollen allergen extracts/vaccines is nearly a hundred years old and their use for immunotherapy is unequaled by any other allergen vaccine. Since Charles Blackley's initial investigations (1) during the 1870s that led to the identification of grass pollen as the cause of his own illness, the study of grass pollen allergens has continued to fascinate botanists, allergists/immunologists and more recently, molecular biologists. In this chapter, the grass family (Poaceae), their ecology, and pollen allergens will be described. Special attention will be given to the molecular characteristics of grass pollen allergens with regard to their cross-reactivities.

Classification and Taxonomy

The grasses belong to the family Poaceae (Gramineae) and are grouped with the sedges, rushes, and other monocots belonging to the order Poales. The family Poaceae is the fourth largest family of flowering plants with more than 600 genera and 10,000 species. The family has historically been divided into two major groups, the pooids and the panicoids, on the basis of the structure of the spikelet, the basic unit of inflorescence (2). The pollen antigens of the pooids and panicoids are immunochemically distinct, as are other characteristics including leaf anatomy, embryo anatomy, and karyotype. These and additional morphological, physiological, biochemical, and cytological comparisons have led to the recognition of up to nine subfamilies and as many as 60 tribes. Most agrostologists today recognize five or six subfamilies although some recognize up to sixteen (3). A taxonomic grouping of common grass genera is presented in Table 1. The classification system is on the basis of that of Watson and Dallwitz (4) with minor modifications. Over 95% of the allergenically important grass species belong to the three subfamilies Pooideae, Chloridoideae and Panicoideae.

THE GRASS FLOWER AND POLLEN

Flowers of the allergenic grasses have obvious characteristics for wind pollination: reduced perianth, small and smooth pollen grains, high pollen-ovule ratio, and feathery stigmas. The flower head, known as the inflorescence (Fig. 1), is made up of spikelets that are highly modified branches consisting of a pair of bracts called glumes. They protect the immature spikelet and a rachilla, on which are borne one to several florets. There is a wide variation in spikelet structure, size and shape, and this is of great value in identification and classification of grasses.

Pollination in grasses is of short duration, and it regularly occurs at a certain time of day or night. The breeding systems of the grasses are extremely varied. Some grasses are cleistogamous (self-fertile) or entomophilous (insect pollinated) and therefore are not allergenically important. Polyploidy is common among the grasses, and hybridization is known to contribute to the adaptation and evolution of many grass groups, especially among the tribe Triticeae, the cereal grasses.

The pollen structure is unique to the family, but they are too uniform to be useful taxonomically (Fig. 2). The pollen is more or less spheroidal to ovoid, 20 to 55 μm in diameter.

Table 1 Taxonomic Relationships Between Common Grasses

Subfamily	Tribe	Genus and species	Common name
Bambusoideae	Oryzeae	<i>Oryza sativa</i> <i>Zizania aquatica</i>	Cultivated rice Wild rice
Arundinoideae	Arundineae	<i>Ehrharta erecta</i> <i>Phragmites communis</i> <i>Cortaderia</i>	Panic, veldt grass Common reed Pampas grass
		<i>Aristida</i> spp.	Three-awns
		<i>Stipa</i> spp.	Needlegrass
		<i>Digitaria sanguinalis</i>	Crabgrass
Panicoideae	Paniceae	<i>Paspalum notatum</i> <i>Panicum miliaceum</i> <i>Panicum virgatum</i>	Bahia grass Common millet Switch grass
		<i>Stentaphrum secundatum</i>	Buffalo grass, Saint Augustine grass
		<i>Eremochloa ophiuroides</i>	Centipede grass
	Andropogoneae	<i>Saccharum officinarum</i> <i>Sorghum halepense</i> <i>Sorghum sudanense</i> <i>Zea mays</i>	Sugar cane Johnson grass Sudan grass Corn, maize
		<i>Bouteloua</i> spp.	Grama grass
Chloridoideae	Chlorideae	<i>Buchloë dactyloides</i> <i>Choris</i> spp. <i>Cynodon dactylon</i>	Buffalo grass Finger grass Bermuda, couch grass
		<i>Distichlis spicata</i>	Salt grass
	Aeluropodeae Eragrosteae	<i>Eragrostis</i> spp. <i>Eleusine indica</i> <i>Tridens flavus</i>	Love grass Goose grass Purpletop
		<i>Bromus inermis</i> <i>Dactylis glomerata</i>	Smooth brome Orchard grass, cocksfoot
		<i>Festuca elatior</i> <i>Lolium multiflorum</i>	Meadow fescue Italian rye
Pooideae	Poaceae	<i>Lolium perenne</i> <i>Poa compressa</i> <i>Poa pratensis</i>	Perennial rye Canada bluegrass Kentucky bluegrass (June grass)
		<i>Agrostis alba</i> <i>Anthoxanthum odoratum</i>	Redtop, bent grass Sweet vernal
	Avenae (including Agrostideae and Phalarideae)	<i>Avena sativa</i> <i>Holcus lanatus</i> <i>Koeleria cristata</i>	Cultivated oat Velvet grass June grass
		<i>Phalaris arundinacea</i> <i>Phalaris canariensis</i> <i>Phleum pratense</i>	Reed canary Canary Timothy grass
		<i>Agropyron repens</i> <i>Elymus</i> spp.	Quack, wheat grass Wild rye
		<i>Hordeum vulgare</i> <i>Secale cereale</i> <i>Triticum aestivum</i>	Barley Cultivated rye Wheat

The pollen grain wall consists of two layers, the exine (outer wall) and the intine (inner wall), and a single germination aperture or pore. Pollen antigens are stored in both the exine and intine walls, most being localized in the intine. A wide range of pollen antigens, including those that are allergenic, undoubtedly have a major role in the recognition of a suitable reproductive partner and thus may be expected to be species specific. Many grass pollen antigens also have wide taxonomic spans. On moistening, exine- and intine-associated components are released into the medium (Fig. 3). The kinetics of antigen release from grass pollen suggests minimal structural compartmentalization as compared to pollen derived from other plant families (5).

Variations in a patient's allergic symptoms during the year depend, in part, on the pattern of seasonal pollen exposure. The expected seasonal levels of grass pollen for a given



Figure 1 Grass plants. (A) Kentucky bluegrass branches; (B) orchard grass; (C) timothy grass.

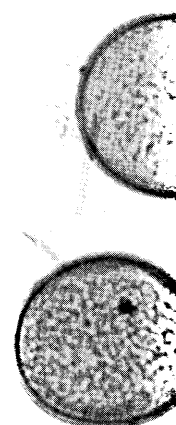


Figure 2 The pollen grains of different grass species they range in size and are characterized by a single germ pore.

geographic location. The Academy of Allergy and Immunology (ACAAI) Pollen and Spore Counting Manual (12) provides whole pollen grain counts and pollen provocation tests. Submicronic pollen provocation tests for asthma attacks are being developed. Sampling and weather (e.g., temperature, humidity) has been identified as a factor in pollen on contact (12), and allergen

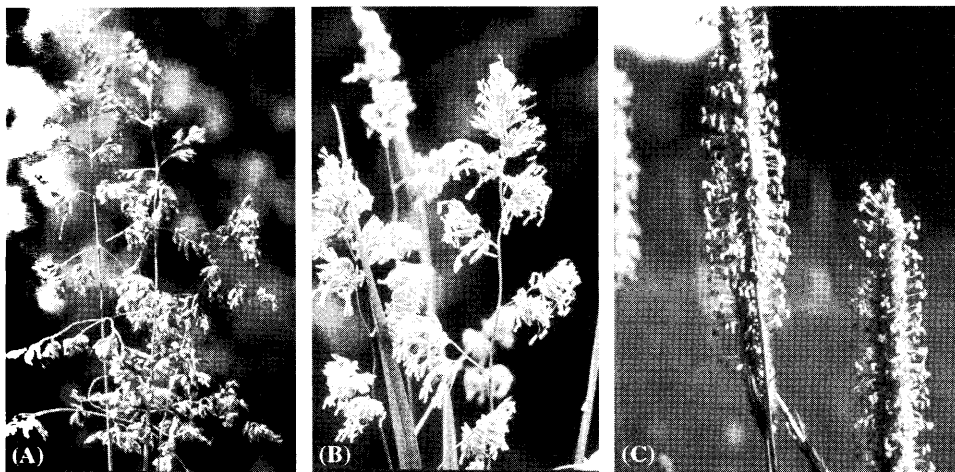


Figure 1 Grass Inflorescence, the arrangement of the flowers on the stem, is illustrated by the three pooids A) Kentucky bluegrass with panicles, a compound inflorescence, bearing flowers along slender, spreading branches; (B) orchard grass with panicles bearing clusters of flowers near the ends of stout branches; and C) timothy grass with spikes, or cylindrical clusters of flowers with no stalks.

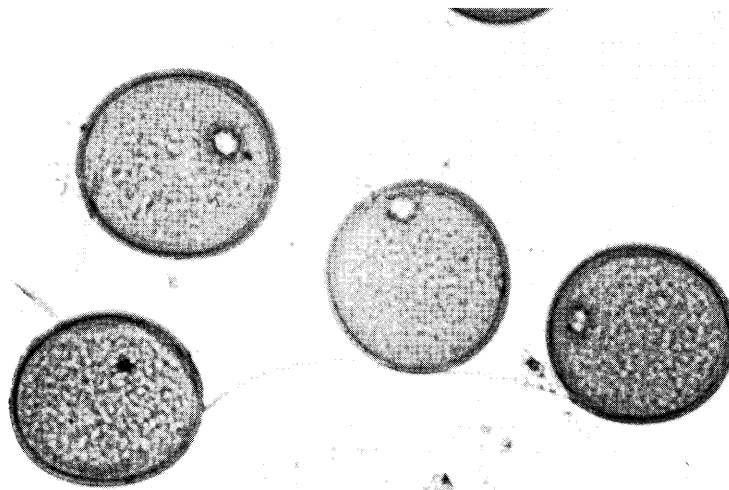


Figure 2 The pollen grains of the grasses are remarkably uniform. They are spheroidal, and in most allergenic species they range from about 20 μm to less than 50 μm in diameter. The exine is thin and has a characteristically granular texture without adornments of any kind. The most distinctive characteristic is the single germ pore, consisting of a small aperture surrounded by a thickened rim of the exine and covered by a transparent membrane.

geographic locality in the U.S. can be obtained from various sources including the American Academy of Allergy Asthma and Immunology's (AAAAI) Aerobiology Committee's Annual Pollen and Spore Reports (6). Grass pollen are most abundant during the spring and summer months and account for a significant portion of the total pollen count during this time. Because whole pollen grains are too large to be respirable, it has been difficult to explain how grass pollen provoke asthmatic symptoms. Several possibilities, including the presence of submicronic particles possessing allergenic activity, have been suggested as the trigger of asthma attacks. The existence of such particles has been confirmed by specialized airborne sampling and immunochemical detection methods (7,8) and has been shown to correlate to weather (e.g., thunderstorms) and epidemics of asthmas (9). A primary source of such particles has been identified as starch granules (0.6–2.5 μm in diameter) that are released from grass pollen on contact with moisture. Other sources, including pollen fragments (10,11), orbicules (12), and allergen-adsorbed aerosols, remain to be investigated.



Figure 3 Ryegrass pollen ruptures after slight wetting with sedimenting mist droplets. The cytoplasmic debris from the ruptured pollen forms an aerosol of respirable particles that are loaded with allergens. *Source:* From Ref. 10.

ECOLOGY AND HABITAT

Grasses occur on all continents, from desert to polar regions and in freshwater to marine habitats and account for about 25% to 35% of the earth's vegetation. The steppes of Eurasia, the prairies and plains of central and western North America, and the Pampa of Argentina represent the most extensive grassland areas of the temperate zone. Less extensive grasslands are found in the velds of South Africa and in Australia and New Zealand. Tropical and subtropical grasslands are located in central Africa and in central South America. In the grasslands, drought, fire and grazing by animals are the major ecological challenges for a plant's survival. The growth tissue in most plants is located at the tip of the leaf or shoot and once clipped, it will not grow back. In contrast, the growth tissue in the grasses is located near the base of the leaf or the shoot, and growth continues even after the grass plant is cropped, burned, or grazed. This and other distinctive features including basal tillering, protection of the flower and fruit within the spikelet, a great diversity of habitats, alternative photosynthetic pathways, breeding systems, and dispersal mechanisms allow them to survive and dominate in areas where other plants cannot.

The distribution of grass species are delimited by conditions of soil, moisture, temperature, exposure, and altitude. Some species are restricted in habitat, being found only in salt marshes or alpine summits. Their geographical range, however, may be extensive. A species found on one mountain range may also be found at the same altitude on another mountain range. Other more tolerant species, such as *Festuca rubra*, can be found in meadows, bogs, marshes and hills of North America, Eurasia, and North Africa.

Seventy percent of the world's farmland is planted in crop grasses with sugar cane (*Saccharum officinarum*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), and maize (*Zea mays*) being the most widely cultivated. Bamboos are a critical part of the economy of many tropical areas because they contribute young shoots for food, fiber for paper, and stems for construction. Grasses are cultivated for livestock feed, erosion control and as ornamentals. Many grasses introduced into cultivation escape and become established over wide areas. Their seeds may be carried long distances in cattle cars as impurities in the seed of crop plants and by birds and insects. Often, they may become troublesome weeds. The turfgrasses are planted to cover lawns, parks, roadsides, cemeteries, golf courses, and sporting fields. Considerable energy is spent maintaining turfgrasses in areas where they would normally not survive. The lawn industry, which accounts for more than a billion dollars of sales of seed, fertilizers, chemicals, paraphernalia, and services, supports the maintenance of grasses in regions that would otherwise be deciduous forests and deserts. Of the hundreds of genera of grasses recognized, only a few are known to cause allergic disease. The major grass species responsible for inducing allergic symptoms are usually those that are cultivated and, therefore, are prevalent where people live (Table 1).

Bambusoideae

The Bambusoideae are the most primitive extant grasses and are associated with forest and aquatic habitats. Bamboos are distributed on the continents of Asia, Africa, North and South America. They are the least understood in terms of their classification and evolution among the

grasses. Bamboos are recorded in the tropics to 120 years elapsing from the first appearance in tropical regions as well as in temperate regions. They are represented among the most primitive grasses by the relative lack of well-developed climbing and advanced climbing and Andes mountain grasses. *Arundinaria tecta* and *Arundinaria donaldsonii* from southern Maryland and the *Bambusa* group, are in the *Bambusa* group. The presence of six stamens in *Zinania aquatica*, are

Arundineae

The Arundineae are the most primitive grasses moved into the open areas. They are distributed in the temperate zone. Of the some 75 genera, *Arundinaria* is the most common in America. This group includes *Arundinaria communis*, which is a common American pampasgrass. They are used as ornamentals in warm climates having adapted to the temperate zone more successful genera.

Pooideae

The temperate zone grasses, consisting of the tribes, defined latitudinal distribution. The center of pooid distribution is in the temperate climates of the open areas, humid and dry tropics, only at high altitudes. Approximately 70% of the grasses are in the middle latitudes. The turfgrasses represent the most common grasses, *Festuca* (fescue) genera along with *Anthoxanthum odoratum* places. The subfamily (wheat), *Secale cereale*

Chloridoideae

The members of the Chloridoideae are the African and Australian grasses, especially the warm nonseasonal rainfall. The chloridoid, compact centers of distribution in Queensland. Their distribution and anatomical features of the Kranz syndrome are widespread throughout. Several species of the range forage grasses

grasses. Bamboos are not allergenically important due to the infrequency of flowering, with up to 120 years elapsing between pollination in many species. The woody bamboos inhabit the tropical regions as well as temperate regions in Asia. The most primitive grasses are represented among the herbaceous bamboos that inhabit the tropical rain forests. Due to the relative lack of wind in their habitats, they have developed animal pollination. The more advanced climbing and herbaceous bamboos have adapted to colder climates of the Himalaya and Andes mountains. The only native bamboos in North America are the two species of *Arundinaria* tecta and *A. gigantea* (giant or switch cane) that grow in moist ground from southern Maryland and Ohio to Florida and Texas. The rices, a herbaceous and mainly aquatic group, are in the Bambusoideae subfamily on the basis of their leaf blade anatomy and the presence of six stamens. The Asian species *Oryza sativa* and the wild rice of North America, *Zizania aquatica*, are the best known species.

Arundineae

The Arundineae are thought to represent the direct descendants of the earliest grasses, which moved into the open savanna ecosystem. This subfamily is a heterogeneous group of unrelated genera and tribes that do not fit into the other relatively well-defined subfamilies. As a group, they are distributed mainly in the tropical and temperate regions of the southern hemisphere. Of the some 75 genera represented in the subfamily, only about five are native in North America. This group includes giant reed (*Arundo*) and the common reed (*Phragmites communis*), which are frequently planted to control erosion. The female plants of the South American pampasgrass (*Cortaderia*), with their large, plumose panicles, are commonly grown as ornamentals in warmer regions of the world. The some 250 species of *Aristida* (three-awns), having adapted to the semiarid habitats of South Africa and northern Mexico, are one of the more successful genera of this subfamily.

Pooideae

The temperate zones are dominated by grasses belonging to the subfamily Pooideae. The major tribes, consisting of about 155 genera, are distributed across the world in relatively well-defined latitudinal belts with the majority of genera found in the northern hemisphere. The center of pooid distribution is the Mediterranean area, and they have adapted to cool and cold climates of the open steppe or meadows. They are virtually absent at low elevations in both humid and dry tropical areas. Species of *Bromus*, *Poa*, *Festuca*, and *Agropyron* can be found only at high altitudes in mountainous regions of tropical latitudes. The pooids account for approximately 70% to 85% of the grasses in Canada and northwestern United States, 40% to 50% in the middle latitudes, and less 15% to 25% in the southern United States. The cool-season turfgrasses representing this subfamily include the genera *Poa* (bluegrasses), *Agrostis* (bent grasses), *Festuca* (fescues) and *Lolium* (ryegrasses). These represent the major allergenic grass genera along with *Dactylis glomerata* (orchard grass), *Phleum pratense* (timothy grass), and *Anthoxanthum odoratum* (vernal grass), which are common in meadows, pastures, and waste places. The subfamily also includes the important cultivated cereals *Triticum aestivum* (wheat), *Secale cereale* (rye), and *Hordeum vulgare* (barley).

Chloridoideae

The members of the subfamily Chloridoideae are well distributed over the North American, African and Australian continents. The chloridoids have adapted to a wide range of ecotypes, especially the warm and arid habitats, with high winter temperatures and summer or nonseasonal rainfall. Over 50% of the grass species in the southwestern United States are chloridoid, compared with less than 10% of the total in the northwestern United States. The centers of distribution are in the savannas of southern Africa and in the open grasslands of Queensland. Their success in the warm, arid environments is due to the distinct physiological and anatomical features of their C_4 dicarboxylic acid pathway of photosynthesis, referred to as the Kranz syndrome. The popular southern turf grass *Cynodon dactylon* (Bermuda grass) is widespread throughout the warmer regions of the world and is a major allergenic species. Several species of *Bouteloua* (grama grass) and *Buchloë* (buffalo grass) are the outstanding range forage grasses and occur widely in the central and western United States.

Panicoideae

The subfamily Panicoideae dominates the humid, tropical to subtropical environments of the savannas of Indochina and Africa as well as the moist New World tropics, especially northeastern South America. Over 75% of the grasses in the Panama Canal Zone are panicoid, 50% in the southern United States, but only about 5% of the species belong to this subfamily in the northwestern United States. The subfamily includes the largest of the grass genera, *Panicum*, with about 600 species distributed throughout the warmer parts of the world and the cultivated species *Saccharum officinarum* (sugar cane) and *Sorghum vulgare* (sorghum). Allergenicity important species include *Paspalum notatum* (Bahia grass), an important forage and erosion control grass in the Gulf Coast states of the United States, and *Sorghum halepense* (Johnson grass), a forage grass and frequently a troublesome weed in the warmer and tropical regions of both hemispheres.

MOLECULAR CHARACTERISTICS AND CROSS-REACTIVITIES OF GRASS POLLEN ALLERGENS

Since the pioneering work of David Marsh and coworkers (13–15) with the perennial ryegrass groups 1, 2, and 3 allergens during the 1960s and 1970s, a number of new allergens have been identified, isolated, and characterized. The International Union of Immunological Societies (IUIS) allergen nomenclature subcommittee's current official list identifies 13 grass pollen allergen groups (Table 2). The techniques of molecular biology and protein chemistry have contributed to the increased knowledge regarding the structure and possible function of grass pollen allergens. Murine monoclonal antibodies raised against specific allergens have been used to define allergenically important and cross-reactive B-cell epitopes as well as to develop specific assays for their detection and quantitation in allergen extracts. Cloning of cDNA and nucleic acid sequencing has accelerated the availability of primary structure data. Recombinant allergen fragments, mutated recombinant proteins, and synthetic peptides have been useful in delineating determinants involved in B- and T-cell recognition. High resolution protein separation and immunoblotting techniques and more recently, the

Table 2 Grass Pollen Allergen Groups

Allergen group	Biochemical name/characteristics	IgE reactivity
1	β -expansins; 27 to 35 kDa major grass pollen allergen produced by every grass species examined so far.	>90%
2	Acidic protein (11 kDa); highly homologous to group 3 and C-terminal portion of group 1 allergens	35% to 50%
3	Basic protein (11–14 kDa); highly homologous to group 2 and C-terminal portion of group 1 allergens	35% to 70%
4	High molecular weight (50–60 kDa) basic glycoprotein; member of the berberine bridge enzyme family, plant pathogen response system.	50% to 75%
5/9	Heterogeneous proteins (27–35 kDa) found in pooid grass species; ribonuclease activity; associated with submicronic cytoplasmic starch particles.	65% to 85%
6	Phl p 6 (12–13 kDa), associated with submicronic cytoplasmic P-particles; homologous to internal Phl p 5 sequences	60 to 70
7	Calcium-binding protein (8–12 kDa) with novel dimer assembly; cross-reactive with birch (Bet v 4), olive (Ole e 3) and rape (Bra r 1) pollen allergens.	10% to 35%
10	Cytochrome c (11 kDa)	<20%
11	Glycoprotein (16–20 kDa); trypsin inhibitor; structurally similar to pollen allergens from olive tree (Ole e 1) and lamb's quarter (Che a 1).	35%
12	Profilin (13–14 kDa); possible association with pollen-plant food cross-sensitization.	20%–50%
13	High molecular weight glycoprotein (45–60 kDa); polygalacturonase; highly susceptible to protease degradation; associated with submicronic cytoplasmic P-particles	30% to 40%
22	Cyn d 22; enolase (42 kDa)	Not determined
24	Cyn d 24; pathogenesis-related protein (21 kDa)	65%

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Group 1 Antigen

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perennial ryegrass allergens have been Immunological Societies es 13 grass pollen in chemistry have the function of grass allergens have been well as to develop ning of cDNA and structure data. synthetic peptides recognition. High ore recently, the

application of proteomic approaches have led to the identification of new allergen groups and the detection of microheterogeneity (isoallergens or isoforms) within the grass allergen groups.

Group 1 Antigens

The grass group 1 allergens are acidic or basic glycoproteins with molecular weights (MW) in the 27 to 35 kDa range and exist in multiple isoallergenic forms or isoforms distinguished by their respective isoelectric points (pIs) and amino acid sequences. Histochemical examination has localized this glycoprotein in the exine and cytoplasm of the pollen grain (16). The protein can account for 6% to 8% of the total extractable pollen protein and has been detected in every grass species examined to date. The complete group 1 amino acid sequences from perennial ryegrass (Lol p 1), timothy grass (Phl p 1), velvet grass (Hol l 1), corn (Zea m 1), Johnson grass (Sor h 1), and Bermuda grass (Cyn d 1) have been determined and the sequences of internal peptide fragments or N-terminal sequences from other several other grass species have been reported (17–21). The degree of glycosylation varies between 2% and 7% and the monosaccharides fucose, arabinose, xylose, mannose and N-acetylglucosamine have been detected. The carbohydrate moiety does not appear to play an important role in the allergenicity of the group 1 allergens although IgE antibodies toward the carbohydrate structures have been detected in a select group of subjects (22,23).

The group 1 allergens belong to a subfamily of structurally related proteins called β -expansins, which are cell wall loosening proteins. Their activity shows specificity to grass cell walls, suggesting that they act on the matrix polymers specific to grasses, for example, glucuronoarabinoxylan or mixed-linked 1,3: 1:4- β -glucan (24). The expansin activity of grass group 1 allergens has been attributed to a papain-like proteinase activity (25–27), but this proposed mechanism of action has been challenged (28). The characteristics of Zea m 1 suggest a specialized role of β -expansins in pollen function (29,30). A function of group 1 allergens is to facilitate growth of the pollen tube and penetration of the ovule to achieve fertilization and thus, breeding success under conditions of pollen competition (31). Structural modeling of the presumed cellulose-binding domain of Lol p 1 suggests a close structural relationship between its cellulose-binding and allergenic properties (32).

Allergens homologous to Lol p 1 have been detected in pollen extracts from all grass species examined to date and in each case greater than 90% of allergic subjects were highly reactive to the respective group 1 allergens. Patient sensitivity to the group 1 allergens has been found to correlate with their sensitivity to the whole pollen extract as measured by both skin test and histamine release assays. Extensive immunological cross-reactivity among the group 1 allergens from taxonomically related grasses is also firmly established. For these reasons, a potency assay on the basis of the group 1 content of grass pollen extracts has been proposed as an approach to grass pollen allergen extract standardization (33).

Two important cross-reactive allergenic determinants or sites have been localized on the pooid grass group 1 allergen molecule with the aid of murine monoclonal antibodies that were selected for their ability to inhibit human IgE binding to grass group 1 allergens. One site has been localized on a 28-mer located at the C-terminus (34) (amino acid residues 213–240) of the molecule and the second site has been localized within amino acid residues 23–35 (35). Continuous B-cell epitopes on Phl p 1 that represent five major IgE-reactive regions of the allergen molecule have been identified by a gene fragmentation approach. The IgE-binding fragments, generated from a random fragment expression library of Phl p 1 (36) and Hol l 1 (20) cDNA, represented regions localized at C-terminus, N-terminus, and in the center of the allergen sequence. The C-terminal portion of the molecule accounts for the majority of the IgE-binding sites of Phl p 1 and they cluster in a sterically oriented manner that may facilitate the cross-linking of effector cell-bound IgE antibodies (37). The importance of correct folding and a stable secondary structure for its allergenic activity is demonstrated by the difficulty to express active, soluble grass group 1 allergens as recombinant molecules. This is not unexpected considering that group 1 proteins possess seven cysteine residues and three disulfide bridges. The replacement of a single cysteine residue at position 77 of Lol p 1 with a serine residues using site-directed mutagenesis was sufficient to reduce the IgE-binding activity by more than 60% (38). Insect-cell expressed Phl p 1 (39) and purified natural Dac g 1 (40) have been shown to possess superior allergenic activity as compared to recombinant molecules expressed in bacteria. A recombinant variant of Phl p 1 introducing a cysteine residue to promote the formation of an additional disulfide bridge yielded a product that was stable, soluble, and

IgE reactivity
>90%
35% to 50%
35% to 70%
50% to 75%
65% to 85%
60 to 70
10% to 35%
<20%
35%
20%–50%
30% to 40%
Not determined
65%

comparable in activity to native Phl p 1 (41). The allergenic epitopes of the group 1 allergen of Bermuda grass (*Cyn d 1*) appear to be different from those defined for the pooid grasses (42) in spite of a 70% to 75% sequence homology. Nonconservative amino acid substitutions in allergenically important regions may explain the lack of cross-reactivity between *Cyn d 1* and the other grass group 1 allergens (19).

A major human T cell determinant has been localized within amino acid residues 191 to 210 utilizing overlapping peptides spanning the entire Lol p 1 molecule (17). Subsequent studies revealed multiple T-cell determinants distributed throughout the molecule including cross-reactive T-cell epitopes shared with grass group 2, group 3, and group 5 allergens (43-47). An immunodominant epitope within residues 126 to 134 was identified in a study employing class II Major Histocompatibility Complex (MHC) tetramers in grass pollen-allergic individuals (48). DR*0401 tetramers loaded with this peptide consistently detected CD4+ T cells expressing a Th2 cytokine profile in allergic but not nonallergic subjects. Nonallergic subjects exhibited very low frequency of such tetramer-specific cells even after allergen stimulation and cell expansion, suggesting a fundamental difference in T-cell responses to group 1 allergens in allergic and nonallergic individuals.

Group 2 Antigens

The grass group 2 allergens are acidic proteins (MW = 11,000) toward which 35 to 50% of grass-allergic subjects are sensitive. The perennial ryegrass group 2 antigens exist in at least 2 immunochemically indistinguishable isoforms, Lol p 2A (pI = 5.0) and Lol P 2B (pI = 5.1-5.3). The complete primary structure of Lol p 2A was determined by peptide sequencing of the purified protein and found to contain 97 amino acids without evidence of glycosylation sites (47). The complete primary structures of Lol p 2, Dac g 2, and Phl p 2 was deduced from the nucleotide sequence of the cDNA encoding the protein and was found homologous to the Lol p 2 sequence (49-51).

Mapping of IgE-reactive epitopes was attempted using a model on the basis of the solution structure of Phl p 2 and recombinant Phl p 2 fragments spanning the entire molecule (52). Only relatively long fragments representing the N-terminal and C-terminal regions of the molecule showed strong IgE reactivity. No reactivity could be detected when synthetic dodecapeptides spanning the complete Phl p 2 sequence were evaluated. These results indicate that grass group 2 IgE epitopes are highly conformation dependent.

The amino acid sequences of group 2 and 3 allergens are also highly homologous. Lol p 2A and Lol p 3 possess 59% identical amino acids and this percentage increased to 67% when similar amino acids are equated. A similar homology was found for Dac g 2 and Dac g 3 with 66% sequence identity and 79% sequence similarity. This sequence homology translates to a high degree of cross-reactivity at the B- and T-cell levels (43,53). The grass group 2 and group 3 allergens also show sequence similarity with the C-terminal portion of the group 1 allergens, although the clinical significance of this has not been thoroughly investigated. It has been suggested that the three allergen groups may share a common origin, with groups 2 and 3 being generated by partial gene duplication of the C-terminal portion of group 1 (54).

Group 3 Antigens

The grass group 3 allergens are basic proteins (MW = 11-14 kDa, pI = 8.9-9.4) with a reported frequency of sensitization of 35% to 70% among grass pollen-allergic subjects. The group 3 allergens from perennial ryegrass, orchard grass and timothy grass pollen have been isolated and characterized at the molecular level (55-57). The complete primary structures of Lol p 3 and Dac g 3 revealed a 92.6% similarity and 84.2% identity and the mature proteins lack cysteine and show no evidence of glycosylation. In spite of this high degree of homology, computer analyses detected differences in their predicted secondary structure and antigenic sites.

Group 4 Antigens

The grass group 4 allergens are high molecular weight basic glycoproteins (MW = 50-60 kDa, pI = 8.6-10.4) with sequence similarity to the berberine bridge enzymes, a member of a flavoprotein oxidoreductase superfamily. The initial report by Marsh found only a 20%

sensitization rate to group 4 allergens from grass pollen. Sensitization rates of 35% to 70% were recently elucidated for group 4 allergens and that deduced from the amino acid sequence of up of carbohydrate sequence. A flavin mononucleotide (FMN) from Bermuda grass pollen was involved in the plant's defense against Dac g 4, reactivity only among two Lol p 4 peptide fragments. Fragmentation destroyed their IgE binding sites (62). to peptides from the p 4-specific monoclonal antibodies. PreadSORPTION with allergens present in the pollen. Together, these findings suggest that group 4 allergens are derived from an allergen from Bermuda grass. 7.5% of the mass, oxidation reduced the allergen's predominant N-linked glycosylation. in that they possess a high degree of this allergen will not cross-reacting carbohydrate

Group 5 Antigens

The group 5 allergens are basic proteins and primary structures of group 5 allergens show a high degree of cross-reactivity with group 3 allergens (27-35 kDa). The traditional protein structure of the group 5 allergens is a monomer. The majority of the group 5 allergens, group 5 allergens are polyclonal antibodies. The allergen is outside of the subfamily. The allergen is homologous to a single chain of a restricted to a single chain of a chloridoid and arum

By using recombinant allergens, investigators have shown that the Lol p 5 molecule is predominantly on a single chain. five discontinuous epitopes in the pollen (Hol l 5), and (79). Conformational changes in a random peptide sequence of amino acids reveal a high degree of suggest either an evolutionary marked difference in grass species. Nevertheless, with significantly more

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between Cyn d 1 and
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sensitization rate toward Lol p 4 among grass-allergic subjects, but other studies suggest that group 4 allergens from timothy, orchard grass, and Bermuda grass may be responsible for sensitization rates of 50%-75%. The complete primary structure of group 4 allergens was only recently elucidated (58,59). On the basis of the reported mass determination of native Phl p 4 and that deduced from the sequence, approximately 10% of its total mass appears to be made up of carbohydrate. Two potential N-glycosylation sites can be identified from the Phl p 4 sequence. A flavin-binding domain, first identified in BG60, the basic 60 kD allergen from Bermuda grass pollen, is consistent with other proteins of the BBE family thought to be involved in the plant pathogen response system (60). Using monoclonal antibodies raised against Dac g 4, related proteins from a various pooid and chloroid grass species, can be detected on SDS-PAGE immunoblots. ELISA inhibition experiments, however, revealed cross-reactivity only among the pooid grasses (61). Allergenic determinants have been localized on two Lol p 4 peptide fragments (MW = 17.4 and 11.0 kDa) by CNBr cleavage of the purified protein. Fragmentation of these Lol p 4 fragments with trypsin or chymotrypsin completely destroyed their IgE-binding capacity, hampering further resolution, and delineation of the allergenic sites (62). A decapeptide sequence of Phl p 4 shows significant sequence similarity to peptides from the major allergen family of ragweed pollen, Amb a 1 and Amb a 2 (63). Phl p 4-specific monoclonal antibody and human IgE antibody binding could be inhibited by preadsorption with Amb a 1. Rabbit and human antibodies directed toward Phl p 4 react with allergens present in various tree and weed pollens as well as vegetables and fruits (64,65). Together, these findings suggest the possibility of common IgE-binding epitopes on grass group 4 allergens and various unrelated pollen and plant foods. Studies with the group 4 allergen from Bermuda grass (66) suggest that the carbohydrate moiety, accounting for about 7.5% of the mass, may be important allergenic determinants of the molecule. Periodate oxidation reduced the IgE-binding activity of the allergen by approximately 50%. The predominant N-linked oligosaccharides of the molecule are unique among plant glycoproteins in that they possess α -(1,3)-linked fucose without any xylose (67). The recombinant forms of this allergen will most likely play a role in delineating allergenically important epitopes and cross-reacting carbohydrate determinants.

Group 5 Antigens (Includes Group 9 Antigens)

The group 5 allergens are a heterogeneous group of proteins with multiple isoforms varying in pIs and primary sequences (68-71). Comparison of the deduced amino acid sequences of the three group 5 allergens shows a high degree of homology (80-90%), which is consistent with the high degree of cross-reactivity observed. Their having similar molecular weights to the group 1 allergens (27-35 kDa for the group 1 and 27-38 kDa for group 5) probably explains why traditional protein fractionation methods based on molecular size failed to establish the identity of the group 5 allergens. The group 5 allergens, together with the group 1 allergens, account for the majority of the IgE-binding reactivity of most grass-allergic sera. In contrast to the group 1 allergens, group 5 allergens have been identified only among the subfamily Pooideae, and polyclonal antibodies raised against group 5 allergens failed to detect cross-reactive antigens outside of the subfamily. Furthermore, Northern analysis with *Poa p V* probes could only identify homologous transcripts among the pooids (72). Thus, it appears that group 5 allergens are restricted to a single subfamily of grasses, and if similar proteins are produced by the panicoid, chloroid and arundinoid grasses, they are immunochemically and genetically unique.

By using recombinant and synthetic allergen fragments, or site-directed mutagenesis, investigators have localized IgE-binding determinants in the central and C-terminal regions of the Lol p 5 molecule (73,74) to both the N-terminal and C-terminal ends of Phl p 5 (75-77) and predominantly on a C-terminal fragment of *Poa p 5* (*Poa p 9*) (78). At least four continuous and five discontinuous IgE-binding sites were localized on the group 5 allergen from velvet grass pollen (Hol l 5), and each were differentially recognized by individual patient IgE antibodies (79). Conformational IgE epitopes of Phl p 5 were mapped using grass-allergic patient sera and a random peptide-phage display library (80). Peptide alignment with the solvent-accessible amino acids revealed at least three sequence sections on Phl p 5a. Taken together, these studies suggest either an extremely heterogeneous human B-cell response to group 5 allergens or a marked difference in the epitope structures of group 5 proteins derived from the different grass species. Nevertheless, a few point mutations in the group 5 sequence can yield mutants with significantly reduced allergenic activity (81,82). In one study, a single deletion (175-198)

in the Ph1 p 5 sequence caused a large reduction in IgE reactivity in a subset of allergic sera and a double deletion (175–198 and 94–113) further reduced IgE reactivity in all sera tested. The hypoallergenic double mutant retained its T-cell reactivity, an important attribute for immunotherapy. A more subtle approach utilizing site-direct mutagenesis involving amino acid exchanges in highly conserved sequence domains may also be good candidates for hypoallergen immunotherapy.

A remarkable characteristic of the group 5 allergens is their association with intracellular starch granules within the pollen grain. The cDNA sequence of Lol p 5 revealed the flanking transit peptide sequences typical of chloroplast-targeted proteins and thus, it has been proposed that the Lol p 5 is synthesized as a preallergen in the cytosol and transported to the amyloplast for posttranslational modification (83). This model may explain the existence of the multiple isoforms and the molecular weight heterogeneity of group 5 allergens isolated from pollen extracts. The size of the starch granules (0.6–2.5 μm in diameter) and their sudden appearance in air samples following rainfall is suggestive of a role in triggering asthmatic reactions. Ph1 p 5, has been shown to possess ribonuclease activity and the homologous group 5 allergens from the other grass species may be expected to possess this activity as well (84). It is interesting to speculate on the role of ribonuclease activity at the level of pollen-stigma interaction: Its release during hydration and stigma contact might facilitate the reproductive responses of the stigma.

T-cell determinants have been localized on Lol p 5 and Ph1 p 5 allergens by generating specific T cell lines or clones and measuring proliferative responses to a series of overlapping group 5 synthetic peptides spanning the entire sequence of the molecule (45,46,85,86). T-cell determinates were spread throughout the allergen molecule. Regions of high reactivity were found in specific patients but they differed among patients. Isoform-specific T cell epitopes were also detected with Ph1 p 5a and Ph1 p 5b fragments. A HLA-DR ligand prediction software (TEPITOPE) identified DR ligands within the Lol p 5a sequence that proved to be novel T-cell epitopes (87). Most of these ligands were clustered at the C-terminal part of a repeated 32-amino acid sequence motif, which occupies about 50% of the Lol p 5 sequence. A similar structural organization is shared by other group 5 allergens suggesting that this repeated motif may be a molecular signature of group 5 allergens. The observed diversity of the human T-cell response and specificity shown by individual patient responses suggest that immunotherapy with allergen peptides is not feasible.

Group 6 Antigens

The group 6 allergens from timothy grass pollen, Ph1 p 6, are polypeptides (MW = 12–13 kDa, pI = 5.2–5.5) toward which a majority of timothy grass pollen-sensitive subjects react (88,89). The group 6 allergens have so far been detected only in timothy pollen extracts. Ph1 p 6 is a pollen-specific protein localized on the polysaccharide-containing wall-precursor bodies or P-particles (88). The amino acid sequence deduced from the cDNA sequence revealed no cysteines and one potential glycosylation site, although no carbohydrate structures were detected (89). The N-terminal sequence of Ph1 p 6 is highly homologous to internal Ph1 p 5 sequences and epitope mapping studies with rPh1 p 6 fragments indicated that the N-terminus of the molecule is required for IgE recognition. Sequence analysis of a cDNA encoding the complete Ph1 p 6 allergen provides evidence for an independent gene family arising from gene duplication. Comparison of the complete Ph1 p 6 and Ph1 p 5 sequences showed only a 55% to 60% match even though the N- and C-termini of Ph1 p 6 showed about a 95% similarity to the internal Ph1 p 5 protein sequence. Both unique and shared epitopes have been identified on Ph1 p 5 and 6 allergens using antibodies raised against Ph1 p 5 and Ph1 p 6, and allergenic crossreactivity has been detected by immunoadsorption studies (90). Epitope mapping studies with rPh1 p 6 fragments indicated that the N-terminus of the molecule is required for IgE recognition.

Group 7 Antigens

The group 7 allergens (MW = 8–12 kDa) from Bermuda (Cyn d 7) and Timothy grass pollen (Ph1 p 7) were identified and isolated from a cDNA expression library using serum IgE from a grass-allergic individuals (91,92). The group 7 allergens belong to a family of Ca^{2+} -binding proteins, characterized by the presence of two potential EF-hand calcium-binding domains (93). Approximately 35% of grass pollen-allergic subjects possessed IgE antibodies toward the recombinant Cyn d 7. In addition, approximately 10% of pollen-allergic patients possessed IgE

antibodies toward to dicotyledonic plants. sequence similarity v v 4 from birch, Aln g from the fungus *Fus* calcium-binding dom structure with an ext similarities with othe and Bra r 1), three EF suggested by molec established and a cro Ca^{2+} -binding domain between Bet v 4 and pollen using RNA allergenic activity m from 16 unrelated ge calcium-binding alle the structure essenti affecting its ability t unlike Bet v 4 and a swapped dimer (97) forms explain the Ig

Group 10 Antigens

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Group 11 Antigens

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Group 12 Antigen

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antibodies toward to group 7-homologous allergens present in pollen of monocotyledonic and dicotyledonic plants. The deduced amino acid sequence of this protein showed significant sequence similarity with a variety of Ca^{2+} -binding proteins, including the pollen allergens Bet v 4 from birch, Aln g 4 from alder, Ole e 3 from olive, Bra r 1 from oilseed rape, and calmodulin from the fungus *Fusarium oxysporum*. A three-dimensional model of Phl p 7 with two calcium-binding domains (EF hands) shows a novel dimer assembly adopting a barrel-like structure with an extended hydrophobic cavity providing a ligand-binding site (94). Structural similarities with other pollen allergens with two EF hands (Bet v 4, Aln g 4, Ole e 3, Cyn d 7 and Bra r 1), three EF hands (Bet v 3), and four EF hands (Jun o 4 and Ole e 8) has also been suggested by molecular modeling studies (95). Cross-reactivity with Bet v 4 has been established and a cross-reactive allergenic epitope was localized to the region representing the Ca^{2+} -binding domain II of the molecule, which shows an 83.3% amino acid sequence identity between Bet v 4 and Cyn d 7. The Cyn d 7 clone hybridized to transcripts in 13 other grass pollen using RNA gel blot analysis, suggesting that homologous proteins with similar allergenic activity may be present in other grass pollens. In addition, pollen extracts derived from 16 unrelated genera exhibited cross-reactivity with Aln g 4 and Jun o 4, suggesting that calcium-binding allergens are widely distributed in pollen from various plants. Disruption of the structure essential for calcium binding reduced the allergenic activity of Phl p 7 without affecting its ability to induce blocking antibodies (96). It is interesting to note that Phl p 7, unlike Bet v 4 and all the other EF-hand proteins appears to occur exclusively as a domain-swapped dimer (97). Presumably, the structural similarities between monomeric and dimeric forms explain the IgE cross-reactivity and that the two forms are allergenically similar.

Group 10 Antigens (Cytochrome c)

Cytochrome c (MW = 11 kDa, pI = 10) from timothy grass, perennial ryegrass, and Bermuda grass pollen have been demonstrated to be allergenic in humans having allergies to the respective pollen. Their importance as allergens on the basis of sensitization rates has not been thoroughly documented. This allergen group has been proposed as a model system for studying the molecular basis of cross-reactivity because a vast knowledge base exists for cytochrome structure and function (98).

Group 11 Antigens

The group 11 allergens are a group of glycoproteins (MW = 16–20 kDa, pI = 5.0–6.0) structurally similar to the Kunitz soy bean trypsin inhibitor, but lack the active site and appear not to possess inhibitory activity. Proteins that are homologous to Lol p 11 have been reported in other grass and non-grass pollen, most notably Ole e 1, the major allergen of olive tree pollen (99–101). This allergen may have eluded detection in conventional immunoblotting techniques using SDS-PAGE under reducing conditions because there are three potential disulfide bridges present that may be required for maintaining the IgE-binding peptide epitopes.

Among individuals with IgE antibodies against grass pollen, approximately 65% possessed IgE antibodies toward Lol p 11 and of these, about 35% possessed IgE antibodies against the carbohydrate moiety. Monosaccharide analysis suggested that *N*-glycan (mannose-type) or arabinoxylan substitutions may represent the IgE-binding carbohydrate group(s). The *N*-glycan structures appear to be involved in IgE binding as is bee venom phospholipase A₂, an allergen with known *N*-glycan IgE-binding epitopes, which is a potent inhibitor of IgE binding to Lol p 11. The recombinant form of Phl p 11, expressed as a soluble fusion protein in *E. coli*, induced histamine release from basophils and skin reactivity in grass pollen-sensitized subjects. The unglycosylated rPhl p 11 showed a reduced prevalence of IgE reactivity among grass pollen-positive sera and little or no cross-reactivity with other members of this allergen family, suggesting its diagnostic utility in identifying the primary sensitizer in allergic individuals.

Group 12 Antigens (Profilin)

Profilin, purified from grass pollen, has been suggested as an important allergen because anti-profilin IgE antibodies can be detected in 20% to 50% of grass-sensitive subjects (102). Because profilin is a highly conserved protein present in all organisms, the potential role of this allergen as a "panallergen" has been proposed (103). The cDNA sequences of timothy grass, Bermuda grass, and birch (*Betula verrucosa*) pollen encoding for the respective profilins are 80%

homologous but, profilins from unrelated sources are much more variable and typically are less than 50% homologous (104,105). Allergic patients with multiple pollen and plant-derived food sensitizations frequently possess IgE antibodies toward profilin. Evidence for both cross-reactive and unique IgE-binding epitopes exist and potential conformational epitopes have been predicted by structural analyses (106). However, the clinical relevance of the cross-reacting or species-specific IgE antibodies could not be established (107).

Group 13 Antigens

The group 13 allergens (MW = 50–60 kDa, pI = 6.0–7.5) have similar molecular weights to the grass group 4 allergens and are difficult to distinguish by one-dimensional SDS-PAGE. This and the finding that group 13 allergens are highly susceptible to proteolytic degradation may explain why they were not identified earlier. Approximately 42% to 75% of grass-sensitive subjects possess IgE antibodies to the group 13 allergens and allergenic proteins homologous to the Phl p 13 have been detected in all grass pollen extracts examined to date. The deduced amino acid sequence from the cloned cDNA of Phl p 13, consisting of 394 residues, indicated homology with pollen-specific polygalacturonases (108,109). IgE-binding epitopes have been mapped to the less-conserved regions of the C-terminus of the molecule and cross-reactivity was restricted to polygalacturonases from grass pollens. The carbohydrate moiety of Phl p 13 has been shown to cross-link IgE receptors on basophils and induce mediator release (23). This has been attributed to the presence of multiple N-glycan epitopes on the molecule. In contrast, Phl p 1, which has at most a single N-glycosylation site, bound IgE antibodies but did not cause mediator release. Phl p 13 has been localized on submicronic particles released from hydrated timothy pollen grains and like Phl p 5, may be considered as environmental markers for grass pollen exposure (110,111).

Group 22 and 24 Antigens

The group 22 (enolase) and group 24 (PR-1) antigens were identified in Bermuda grass pollen extract using a proteomic approach combining two-dimensional electrophoretic separations, immunoblotting, MALDI-MS, LC-MS/MS, and bioinformatics to identify these novel pollen allergens (112). Both proteins have been purified from whole Bermuda grass pollen extracts and shown to possess allergenic activity on the basis of histamine release or skin test responses in allergic patients. Cyn d 24 is a glycoprotein (MW = 19–21 kDa, pI = 5.9) structurally similar to the plant pathogenesis-related proteins (PR-1). Sequence identity ranged from 45% to 50% with PR-1s from barley, wheat, maize, and rice (113). The carbohydrate moiety, which makes up about 6% of the total weight of the glycoprotein resembles the structure possessed by Cyn d 4 (BG60): An L-fucose α -(1,3)-linked to an N-acetyl glucosamine without xylose linked to the branching mannose. As with Cyn d 4, there is evidence that the carbohydrate moiety is involved with IgE binding.

GRASS POLLEN ALLERGEN CROSS-REACTIVITY

Grass-allergic subjects almost always display multiple grass pollen sensitivities. Because many grass species coexist in the same geographical area, simultaneous sensitization to pollen from multiple grass species is expected. Various approaches have been used to investigate grass pollen allergen cross-reactivities and most of these studies support the hypothesis that sensitization to one grass pollen species leads to multiple pollen sensitivities. RAST and ELISA inhibition assays have revealed extensive allergenic cross-reactivity among taxonomically related grasses (114). Much data have been generated using whole pollen extracts but more precise information about cross-reactivity, i.e., relative affinity, can only be obtained by using individual specific allergens. Structural details of homologous grass pollen allergens may also reveal potential cross-reactive epitopes (115,116). These studies may be on the basis of the primary amino acid sequence data or on more sophisticated 3-dimensional structural data of surface exposed residues.

The pattern of allergenic cross-reactivity among the pollen species follows closely their taxonomic relationships (Fig. 4). All studies found the highest degree of allergenic cross-reactivity among pollen extracts derived from grasses of the same subfamily. Martin et al. (117), using a human serum pool from allergic North American subjects with pollen extracts

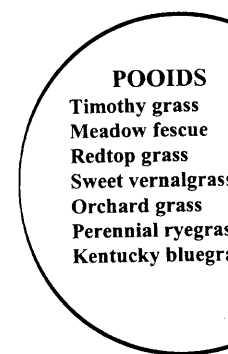


Figure 4 A Venn diagram showing the overlap of allergenic cross-reactivity between different grass pollen groups. The left circle represents the POOIDS group (Timothy grass, Meadow fescue, Redtop grass, Sweet vernalgrass, Orchard grass, Perennial ryegrass, Kentucky bluegrass) and the right circle represents the Cyn d 4 group (Bermuda grass, Cyn d 4, Cyn d 24). The overlapping area indicates cross-reactivity between these two groups.

from the three subfamilies (sweet vernal, redtop, Bermuda grass, and little or no cross-reactivity was observed. However, in reciprocal assays, grass pollen reactivity toward the pooids (perennial ryegrass, common reed), and chloridoids. In a reciprocal assay, cross-reactivity was directed toward the arundinoid, Cyn d 4, sufficient for in vivo possible allergenicity.

The available allergens has all derived from the same. Of particular interest is the molecule (aminoglycoside) group 2 and group 24 associated with the three allergens Lol p 1, Lol p 2, and Lol p 3 (43,53). Some groups and the nature suggest that

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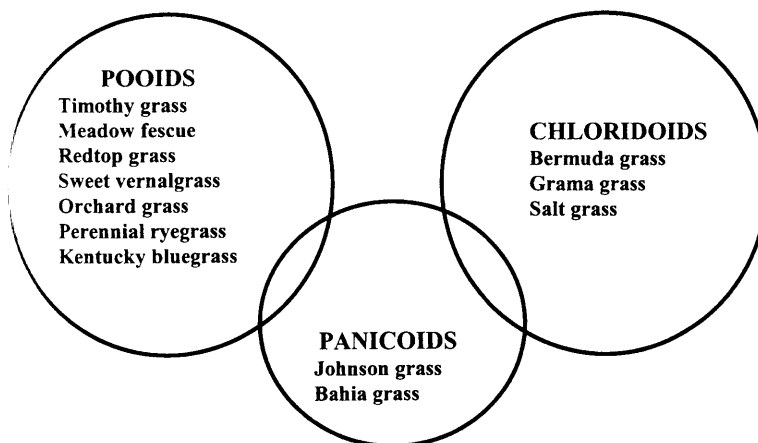


Figure 4 A Venn diagram representing cross-reactivity grouping of major allergenic grasses based on their taxonomic and immunological relationship. The grass species within each subfamily group are highly cross-reactive and are difficult to differentiate immunologically. The two groups pooids and chloridoids are allergenically distinct and require separate diagnoses and immunotherapies. The panicoids, Johnson and Bahia grass, are allergenically cross-reactive with both the pooids and chloridoids.

from the three subfamilies Pooideae (brome, meadow fescue, perennial ryegrass, timothy, sweet vernal, redtop, Kentucky bluegrass, and Western wheatgrass), Chloridoideae (salt grass, Bermuda grass, and grama grass) and Panicoideae (Bahia grass and Johnson grass), detected little or no cross-reactivity between pooid and chloridoid pollen while the panacoid grasses showed moderate cross-reactivity with both the pooids and chloridoids. In a study using IgE antibodies from Australian subjects and selected for reactivity to Lol p 1 and Lol p 5, no cross-reactivity was observed between ryegrass and Bahia or Bermuda grass pollen allergens. However, in reciprocal experiments using antibodies selected for reactivity to Bahia pollen allergens, grass pollens from ryegrass, Timothy, Johnson grass, and Bermuda were cross-reactive (118). Gonzalez et al. (119), employing sera from European subjects selected for reactivity toward each pollen group, detected little or no allergenic cross-reactivity between the pooids (perennial ryegrass, timothy and cultivated rye) and Bermuda grass. *P. communis* (common reed), an arundinoid, showed moderate cross-reactivity with both the pooids and chloridoids. In a study with 209 individual sera with reactivity toward grass pollen, extensive cross-reactivity was detected among 14 different species of pooids with the highest responses directed toward *P. pratensis*, *F. rubra*, *P. pratense*, and *D. glomerata*. Excluding *P. communis*, an arundinoid, *C. dactylon*, a chloridoid, and *Z. mays*, a panicoid, any one grass species was sufficient for in vitro diagnosis of grass pollen allergy (120). No studies have examined possible allergenic cross-reactivities between arundinoid and panicoid pollen.

The availability of purified grass pollen allergens, allergen fragments, and recombinant allergens has allowed for more refined studies that detect cross-reactivity among allergens derived from the same species as well as among homologous allergens from different species. Of particular interest is the strong homology between the C-terminal end of the group 1 molecule (amino acid residues 145–240) and the entire 97-amino acid sequence of the grass group 2 and group 3 allergens. The human immune response to the three ryegrass allergens is associated with histocompatibility leukocyte antigen (HLA) DR3 and concordant reactivity to all three allergens is common. These observations may be explained by cross-reactivity among Lol p 1, Lol p 2, and Lol p 3, which has been detected at both the B-cell level and at the T-cell level (43,53). Some human T-cell clones are reactive to the purified protein of the three allergen groups and the group 5 allergen. Stretches of homologous segments with an amphipathic nature suggest the presence of structural similarities that may account for this cross-reactivity.

The cross-reactive nature of the ubiquitous protein profilin is illustrated by the serological cross-reactivity between grass pollen profilins and profilins from other pollen and vegetable foods (121). Human anti-profilin IgE antibodies have been shown to cross-react with almost all plant profilins. The presence of highly conserved structures among plant

profilins may explain the reports of coincident oral allergies to fruits and vegetables eaten by grass-allergic subjects (102,122,123) as well as allergenic cross-reactivities occasionally detected between unrelated pollen. The group 4 and group 7 allergens may also be useful diagnostic markers to identify patients with multiple sensitizations caused by cross-reactivity. For example, grass group 4-related proteins have been identified in unrelated plant foods including peanut, apple, celery, and carrot root as well as mugwort and birch pollen. Approximately 20% of polysensitized individuals possess IgE antibodies toward calcium-binding pollen proteins. Among the calcium-binding proteins, Phl p 7 contained most of the relevant IgE epitopes in the population studied and may be a useful molecule for detecting sensitization to this group of cross-reactive pollen allergens.

Cross-reactive carbohydrate determinants of grass pollen allergens have also been implicated in serological cross-reactions among a variety of pollen and vegetable foods (124-126). The β (1-2)-xylose- and α (1-3)-fucose-containing glycans on glycoproteins from several plants, mollusks, and insects have been shown to be highly cross-reactive. The clinical significance of anti-carbohydrate IgE antibodies has not been established and the role of CCD in allergic sensitization remains to be explored. In this regard, the grass group 11 allergens, Cyn d 4 (BG60), and Cyn d 24 may present a unique opportunity to investigate the role of both carbohydrate and peptide epitopes in allergenic cross-reactivity among glycoproteins from related and unrelated sources. The presence of conserved amino acids and cysteine positions in the primary structure of Lol p 11 suggests homology with pollen glycoproteins from maize, rice, tomato, olive tree, and privet.

Since grass pollen immunotherapy was pioneered by Freeman and Noon (127) almost a century ago, the specificity of grass immunotherapy has been questioned. Freeman advocated the use of only extracts/vaccines from timothy grass pollen for the diagnosis and treatment of grass allergy in Great Britain as did Cooke and Vander Veer (128) in the United States. Leavengood et al. (129) selected a group of patients showing multiple sensitivities to pooid, panicoid, and chloridoid grasses and treated them with vaccines prepared only from timothy and Bermuda grass pollen. The treatment significantly reduced the skin test responses to all of the grass pollen, suggesting that treatment with the two grass pollen vaccines may be sufficient for effective treatment in these grass-allergic patients. The current practice parameters for allergen immunotherapy established by both the American Academy of Allergy Asthma and Immunology and the American College of Allergy Asthma and Immunology specifically states that information regarding allergen cross-reactivity should be used in the selection of relevant allergens for immunotherapy because limiting the number of allergens in a treatment vial may be necessary to attain optimal therapeutic doses for the individual patient (130). The molecular and clinical evidence for cross-reactivity among grass pollen allergens supports the premise that effective diagnosis and immunotherapy can be accomplished with a limited number of grass pollen extracts/vaccines. The use of representative extracts/vaccines from the major grass subfamilies appears to be a reliable strategy, and the selection of the species should be based on their prevalence. For example, timothy grass (or any of the pooids), Bermuda grass and Johnson grass, which represent the three major allergenic grass subfamilies should be sufficient for clinical practice.

CONCLUSION

Grasses are ubiquitous and their pollens are important aeroallergens in most parts of the world. Only a few grass species have been positively identified as important sources of allergens, but less conspicuous grass species may add to the aeroallergen load due to their cross-reactivity. The degree of allergenic cross-reactivity tends to correlate with their taxonomic grouping and the treatment of grass allergy with vaccines derived from representative species have been shown to be efficacious. Cross-reactivities between homologous proteins from different grass species and their clinical relevance have long been established. There is evidence of cross-reactivities between grass pollen allergens and proteins derived from diverse plant sources including other pollen, fruits, and vegetables. Clinical investigations to establish the relevance of such cross-reactivities are still needed.

New immunochemical and molecular biological approaches to the study of grass pollen allergens have greatly increased the knowledge about this important group of pollen allergens.

Due to their worldwide prevalence, grass pollen allergens have been identified and characterized. More than 100 allergenic proteins have been assembled more than 100 different grass pollen allergen agencies are funding research on grass pollen allergens. Members of the grass pollen allergen community are exchanging genetic information via the Internet. For example, the completion of the human genome project (134) suggests that at least 100 allergen genes (135) will be identified and characterized. The major grass allergens are being characterized both the B-cell and T-cell epitopes of allergen molecules and their immunological activities. This information will be used to develop new diagnostic and therapeutic approaches from other environmental allergens. A better understanding of allergen cross-reactivity is needed.

SALIENT POINTS

- Grasses are the most important sources of aeroallergens.
- Of the hundred species of grasses, only a few are allergenically important.
- The pooids are the most important grasses and are prevalent in the United States, especially in the southwestern United States. The major allergenic grasses include timothy, Bermuda, and Johnson grass.
- Allergic symptoms are caused by the release of histamine and other mediators from mast cells. The size of the allergen molecule is implicated in the allergic response.
- Eleven groups of grass pollen allergens are recognized across multiple grass species.
- Grass-allergic patients may be sensitized to multiple allergens at a given time.
- Allergenic cross-reactivity is produced by homologous proteins from different grass species.
- The presence of cross-reactivity is a determinant of the allergic response to reactions to grass pollen allergens in patients.
- Diagnosis and treatment of grass allergy are represented by the use of representative grass pollen allergens. This approach is effective in the treatment of grass allergy.
- Advances in the study of grass pollen allergens will increase our understanding of allergen cross-reactivity and the development of new diagnostic and therapeutic approaches.

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vegetables eaten by occasionally detected be useful diagnostic cross-reactivity. For related plant foods and birch pollen. es toward calcium- contained most of the molecule for detecting

ns have also been able foods (124-126). from several plants, nical significance of of CCD in allergic ns, Cyn d 4 (BG60), h carbohydrate and ated and unrelated primary structure of ato, olive tree, and

oon (127) almost a Freeman advocated es and treatment of the United States. sitivities to pooid, only from timothy responses to all of s may be sufficient ce parameters forergy Asthma and specifically states ection of relevant treatment vial may 80). The molecular ports the premise mited number of s from the major species should be . Bermuda grass milies should be

most parts of the rtant sources of ad due to their elate with their s derived from between homol- have long been ns and proteins etables. Clinical ded. of grass pollen pollen allergens.

Due to their worldwide importance, grass allergens are a subject of great interest among researchers from virtually every continent and more than 100 grass pollen allergen structures have been identified, purified, and sequenced. Two groups independently sequenced and assembled more than 90% of the rice (*Oryza sativa*) genome (131,132) and the U.S. government agencies are funding a \$40 million effort to sequence the corn genome (133). Because the members of the grass family are closely related and their genomes share extensive synteny, this genetic information will undoubtedly have a great impact on grass pollen allergen research. For example, the complexity of grass group 1 allergen genes and their pattern of expression (134) suggests that attempts to silence their expression as has been attempted with the group 5 allergen genes (135) will not be successful. Only a few minor grass pollen allergen groups are yet to be identified and it is expected that in the next few years the molecular structure of all of the major grass allergen groups will have been established. The human immune responses at both the B-cell and T-cell levels are being studied to define relevant structures on the grass allergen molecules and novel diagnostic and therapeutic approaches based on their immunological activities are in progress. Together with advances being made with allergens from other environmental sources, the study of grass pollen will contribute to a better understanding of allergic responses to these ubiquitous allergens.

SALIENT POINTS

- Grasses are ubiquitous and grass pollen allergens are of worldwide importance.
- Of the hundreds of grass genera and thousands of species, only a small number are allergenically important. Most of these species are cultivated.
- The pooids account for most grass species in Canada and northwestern United States and are prevalent in the cooler regions of the world; the chloridoideae are well established especially in the warm and arid habitats with high winter temperatures such as the southwestern United States; and the panicoids dominate the humid tropical environments including the southern United States and northeastern South America.
- Allergic symptoms depend on pollen exposure. Particles that are significantly smaller than the size of grass pollen grains and capable of entering the lower airway have been implicated as major causes of allergic reactions.
- Eleven groups of structurally related allergens have been identified and characterized across multiple grass subfamilies.
- Grass-allergic patients often display multiple sensitivities. Simultaneous sensitization to multiple species are expected on the basis of the numerous grass species pollinating at a given time and place and on the high degree of cross-reactivity among them.
- Allergenic cross-reactivities have been documented among homologous allergens produced by taxonomically related grasses and between allergens produced in a single grass species.
- The presence of conserved structures among the proteins and various carbohydrate determinants of pollen and vegetable foods is consistent with coincident allergic reactions to fruits and vegetables as well as unrelated pollen in grass pollen-allergic patients.
- Diagnosis and immunotherapy with a limited number of grass allergen vaccines representing the subfamilies Pooideae, Chloridoideae and Panicoideae may be effective in most grass-allergic patients.
- Advances made with the rice and corn genome projects should be leveraged to increase understanding of the structure, expression, and function of grass pollen allergens.

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8 Weed

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INTRODUCTION

Pollens derived from allergenic proteins in midsummer and extend immunotherapy with. There are many other are by far the most molecular biological constituents of different of their allergens and the integration of molecular biochemical and clinical

TAXONOMY OF WEED

Of the families of weeds allergy. As for other comprehensive list of family and genus), of an aerobiologist show the surrounding geographic American Academy Pollen Hotline, 1-800 and Immunology, 61

WEED AND POLLEN

Although there are Some of the important features of short ragweed subdivided into three appearance. The pollen has been disturbed. They are 4 to 5 ft, producing ragweed (*A. trifida*) from blades of the leaf canopy grows above the ground. Western ragweed is have inconsistently of Western giant ragweed tendency to branch ragweed has slender