



Aeroallergens and pollinosis: Molecular and immunological characteristics of cloned pollen allergens

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Abstract

Affecting almost twenty percent of the population in industrialized countries, allergic diseases such as asthma and seasonal rhinitis result from the development of immediate hypersensitivity to otherwise innocuous components of the environment. Pollen, the male gametophyte of flowering plant species, is one of the most predominant sources of environmental allergens, and a significant cause of allergic diseases. This review discusses the nature of pollen proteins as allergens, their effect on the human immune system and their mode of environmental transmission, including effects of the interaction between pollen aeroallergens and air pollution. The influence of cross-reactive pollen allergens to the incidence of food sensitivities is also discussed. Flowering plant species that possess allergenic pollen, identified with allergens cloned from these species, are also discussed.

Introduction

Allergy to the pollen of flowering plant species significantly impacts on the health of people in many parts of the world. With up to 20% of the populations in industrialized countries suffering from pollen allergy, much research has been conducted to identify allergenic pollen species and the allergy-causing components present in their pollen.

Pollen contains many proteins but only a small portion of these has the ability to interact with the human immune system to cause allergic symptoms. Certain biochemical properties of allergenic proteins contribute to this interaction, including localization of allergens within pollen, rapidity of solubilization upon pollen hydration and recognition of components of the proteins by the human immune system (Knox and Heslop-Harrison, 1970; Singh et al., 1991; Arnon and Van Regenmortel, 1992; Vrtala et al., 1993a).

Genes and cDNA sequences encoding allergenic proteins from the pollen of many grass, tree, weed and crop species have subsequently been cloned

and sequenced. Cloning and recombinant expression of pollen allergens has allowed determination of molecular and immunological characteristics of these proteins including identification of the sites that interact with the human immune system and therefore lead to the development of allergy symptoms. Structural similarities with functional proteins and enzymes have been identified for a number of pollen allergens, suggesting clues as to the function of these proteins. Cloning of pollen allergens has also allowed identification of immunologically cross-reactive proteins in pollen from taxonomically related and distant plant species, and plant-derived food extracts. These observations have helped to explain the incidence of sensitivity to pollen and foods derived from distantly related plant species often displayed by pollen allergic patients.

The mode of environmental transmission of pollen can influence the level and incidence of exposure to allergens by sensitized individuals, with wind pollinated plant species posing a greater risk of exposure than insect pollinated species (Smart et al., 1979;

Solomon, 1984; Jimenez et al., 1994). Furthermore, the interaction between aeroallergens and particulate and gaseous air pollutants, and the presence of allergen-containing, paucimicronic particles in the atmosphere have also recently been shown to be associated with allergic sensitization (Behrendt et al., 1995; 1997).

Allergic diseases

Atopy, defined as the ability to produce IgE antibodies to common allergens, demonstrable by skin prick test reactivity, is clinically manifested as allergic diseases such as asthma, rhinitis, eczema and food and animal sensitivities. With up to 25% of the populations in industrialized countries suffering these diseases, the impact of allergy on society is significant. Of the diseases, allergic rhinitis (known as hay fever) has the highest incidence with 10–20% of the population suffering from this disorder; 5–10% exhibit asthmatic symptoms while 1–3% experience food allergies (Durham and Church, 2001). Whilst food allergy and eczema are often predominant in infancy, asthma and rhinitis often may not develop until late in childhood or early adulthood. Pollen allergy is also typically a seasonal disease often dependent upon the flowering season of the plant to which an individual is pollen-sensitive. However, polysensitized, pollen-allergic individuals may suffer symptoms of hay fever almost year-round.

Pollen allergy

The most predominant source of allergens in the outdoor environment is pollen, the male gametophyte of flowering plants. Symptoms of sensitivity to allergens in pollen typically develop upon contact of pollen with the mucosal surfaces of the eyes, nose and throat of sensitized individuals (Bush, 1989). This typically results in the development of conjunctivitis, nasal congestion, sneezing and irritation of the eyes, nose and throat. Hay fever is often the result of inhalation of whole pollen or large pollen particles (Wilson et al., 1973). Smaller pollen particles and allergen-carrying components can often be inhaled into the lower airways where they induce the development of asthmatic symptoms in sensitive individuals, including breathlessness, mucosal inflammation and hypersecretion, muscle hyper-reactivity and anxiety (Stewart and Holt, 1985).

Identified allergenic proteins

The vast majority of pollen allergens so far identified are proteins, both glycosylated and non-glycosylated, and range between 10 to 70 kDa in molecular weight. Some possess actin-binding properties, others the ability to bind calcium ions. For most of these allergens, biological function is undetermined but is almost certainly not a significant causative factor in allergenicity. Besides interacting with the immune system, most of these proteins are otherwise innocuous. Furthermore, they do not have common structural features that indicate they have the ability to interact with the immune system, and as proteins they are not recognizable as allergens without interaction with IgE antibodies and the immune system.

Allergens are named and classified according to their level of immunological reactivity (King et al., 1994). A protein is defined as a major allergen when it causes an allergic reaction in at least 50% of individuals sensitive to the particular species from which the allergen is derived. Therefore, minor allergens are those that cause allergy in less than 50% of sensitive individuals. The identifying names of allergenic proteins are derived from the name of the species from which they were isolated. For instance, allergens derived from the grass *Lolium perenne* (rye grass) are called 'Lol p' allergens; allergens from the tree *Betula verrucosa* (birch) are known as 'Bet v' allergens. Furthermore, these proteins are classified into groups based on similar structural properties and immunological cross-reactivity with other allergens from similar sources. Thus, the group 1 pollen allergen from rye grass is identified as 'Lol p 1' and shares significant amino acid homology and cross-reactivity with the group 1 pollen allergens from other grass species. Likewise, the group 1 allergen from birch is identified as 'Bet v 1' and shares significant amino acid homology and cross-reactivity with the group 1 allergens from other *Fagales* tree species. With the exception of certain allergens (as described later), structural properties and immunological cross-reactivity are generally limited to taxonomically linked species and thus in general, for example, the group 1 allergens from grass pollen and tree pollen are not cross-reactive.

Pollen proteins as allergens

The allergenic properties of pollen are not part of its biological function but an unfortunate coinci-

dence brought about by certain biochemical properties of the pollen and an interaction of certain components with the human immune system. The allergens within pollen are exposed to the immune system only upon solubilization or release from pollen grains. A number of studies have shown that hydration of pollen results in the rapid release of allergenic proteins into the surrounding medium (Knox and Heslop-Harrison, 1970; Vrtala et al., 1993a). Most of the allergenic proteins in the pollens of *Betula verrucosa* (birch) and *Phleum pratense* (timothy grass) are released within 10 minutes of hydration, with the most allergenic proteins being released after only 30 seconds (Vrtala et al., 1993a). These studies have demonstrated the rapidity at which allergenic proteins may be released from pollen grains and the ease with which they come in contact with the human body. The less allergenic proteins are released more slowly or in smaller amounts than the major allergenic proteins (Vrtala et al., 1993a). Furthermore, non-allergenic proteins seem to be retained in the pollen and are released only when harsh chemical or mechanical extraction methods are applied (Vrtala et al., 1993a).

Pollen contains many proteins, but only a relatively small number of these exhibit allergenic properties. Allergenic proteins are characterized solely on their ability to induce an allergic response in sensitive individuals – no common structural or functional feature of these molecules has been identified to explain this ability. Two significant factors contribute to the allergenicity of pollen proteins. Firstly, proteins must be rapidly solubilized from pollen upon contact with moist surfaces. Pollen is generally in contact with the body's mucosal surfaces for a short period of time since mucociliary action induces sneezing or carries the irritants down the back of the throat to be degraded (Bridger and Proctor, 1971). Secondly, these proteins must be processed and presented in such a way that they are recognized by components of the human immune system. Each allergen possesses regions known as epitopes, recognized by specific components of the human immune system – either immunoglobulin E (IgE) antibodies or T-cell lymphocytes (Arnon and Van Regenmortel, 1992). The regions that are identified as IgE or T-cell epitopes are classified as such based solely on their ability to be recognized by IgE antibodies or T-cell lymphocytes. They do not have common structural features that indicate they have the ability to interact with the immune system, and they are not recognizable without IgE or T-cell interaction. Specific recognition of an allergen by IgE antibodies

leads to the development of the symptoms of allergy (Sutton and Gould, 1993). Recognition of an antigenic determinant from an allergen by T-cells leads to an interaction with phagocytic cells resulting in destruction of the foreign molecule (Roitt et al., 1998).

Localization of allergens within pollen

Pollen grains have a cytoplasm containing vegetative and generative nuclei, and various organelles, including endoplasmic reticulum and mitochondria, with a grain wall composed of an intine (internal cellulose wall) and an exine (outer sporopollenin wall) (Knox, 1984). While this wall provides a barrier to contain the pollen components, apertures in the exine allow for the emergence of the pollen tube upon germination and movement of water and proteins upon hydration. A major allergen from rye grass pollen, Lol p 5, is localized within intracellular starch granules (Singh et al., 1991). Upon contact with rainwater, osmotic shock has been shown to burst pollen grains releasing these granules into the atmosphere. A fifty-fold increase in the atmospheric concentration of these granules is measurable on days following rainfall (Suphioglu et al., 1992). Allergens in such granules are more readily inhaled due to the ease with which such small granules may enter the airways, and thus, are more readily encountered by sensitive individuals in this form. In comparison, the group 1 allergen from rye grass pollen, Lol p 1, was found localized in the cytosol (Taylor et al., 1994). The major pollen allergen from timothy grass, Phl p 1, has been shown to occur in the cytoplasm and intine, but is absent from the exine (Behrendt et al., 1997). The location of two other pollen allergen from timothy grass have also been demonstrated. Phl p 4 is reportedly associated with cytoplasmic matrix sites and intracellular starch granules (Fischer et al., 1996), while Phl p 6 has been found to localize with P-particles in the cytoplasm of the pollen (Vrtala et al., 1999).

Plants with allergenic pollen and cloned pollen allergens

Pollen is present in the atmosphere all year round. Whilst allergenic pollen species may account for only 20 to 30% of the total annual pollen catch, they represent the predominant species during the respective flowering seasons. Trees begin flowering

in winter and are the major source of airborne pollen allergens in northern Europe (D'Amato et al., 1998). Grasses flower through spring and summer and are the major source of airborne pollen allergens in Australia, California, many parts of Europe and other cool-temperate climates (Smart et al., 1979; Spieksma, 1990; D'Amato et al., 1998). Weeds, such as those of the *Ambrosia* (ragweed) and *Artemisia* (mugwort) genera, flower in late summer and are the predominant source of pollen allergens in North America and eastern Europe (King et al., 1976; D'Amato et al., 1998). In different regions of the world the major allergenic pollen species may originate from any of these sources. Allergenic pollen has been identified in many flowering plant species including grass, weed, tree and crop species. Besides common biochemical functions or amino acid conservation (for example, calcium binding domains), similarity between pollen allergens is limited to within plant families – the grass, tree, weed and crop pollen allergens generally do not share sequence homology or immunological cross-reactivity.

Allergenic grass species

Grasses, monocotyledonous, non-woody plants, are typically grown as ground cover and pasture plants but may also have a weedy nature, inhabiting disturbed ground. Grasses are long-lasting, robust plants, a characteristic that undoubtedly contributes to their widespread distribution. With over 1000 different species found on all populated continents of the globe, grasses contribute significantly to pollen allergy in many parts of the world. The most allergenic grass species, all belonging to the subfamily *Pooideae*, are *Phleum pratense* (timothy grass), *Poa pratensis* (Kentucky bluegrass), *Dactylis glomerata* (orchard grass), *Festuca elatior* (meadow fescue), rye grass and *Holcus lanatus* (velvet grass). In the cool-temperate climates of California and parts of Europe, rye grass pollen is responsible for allergy in more people than any other pollen source (Spieksma, 1990; D'Amato et al., 1998). Rye grass is also the major contributor to the level of pollen in the atmosphere above the cool-temperate conditions of southern Australia (Smart et al., 1979). Kentucky blue grass is the major contributor to the level of grass pollen allergens in the atmosphere above North America, while timothy grass is the major contributor in Europe (Spieksma, 1990). The pollen of *Cynodon dactylon* (Bermuda grass) and *Sorghum halepense* (Johnson grass), respectively, are

the major sources of grass pollen allergens in tropical and sub-tropical climates.

Some of the most widely cultivated crops are grass species – *Hordeum vulgare* (barley), *Triticum aestivum* (wheat), *Avena sativa* (oats), *Oryza sativa* (rice) and *Zea mays* (maize) have been bred for high seed yield. The pollen of these grasses, along with *Secale cereale* (rye) and *Triticosecale cereale* (triticale) have been shown to house allergenic proteins (Astwood et al., 1995).

Cloned grass pollen allergen genes

Pollen allergen genes have been cloned from many commonly occurring grass species including certain crop plants (Table 1). Of the grass pollen allergens cloned, the group 1 and group 5 proteins are the most allergenic with approximately 90% of all grass pollen allergic patients showing concordant immunoreactivity to both groups of proteins (Freidhoff et al., 1986). Due to this, many studies have been conducted to try to determine the molecular and immunological characteristics of these proteins.

Group 1. The group 1 grass pollen allergens are glycosylated proteins of approximately 28–35 kD in mass (Freidhoff et al., 1986). Although homologues have been found in all grass pollen species tested (Matthieson and Lowenstein, 1991; Avjioglu, 1992; van Ree et al., 1992a; Avjioglu et al., 1993), the genes from only eight group 1 grass pollen allergens have been cloned (Table 1). The group 1 pollen allergens isolated from the grasses in the *Pooideae* subfamily share over 90% amino acid homology and possess seven strictly conserved cysteine residues. Recombinantly expressed group 1 grass pollen allergens have been shown to maintain a level of IgE reactivity similar to the native allergens (Laffer et al., 1994; Boutin et al., 1997). When expressed in the yeast *Pichia pastoris*, recombinant Cyn d 1 showed an IgE binding capacity similar to the native allergen (Smith et al., 1996).

To obtain clues to immune recognition of the group 1 grass pollen allergens, the IgE and T cell epitopes of some of these have been mapped. Esch and Klapper (1989) mapped a major IgE-binding epitope to within the last 28 amino acids of the C-terminal region of five grass group 1 pollen allergens, including Lol p 1, Fes e 1, Agr a 1, Poa p 1, Ant o 1. Two internal regions, corresponding to amino acids 25 to 40 and 151 to 168 of Lol p 1, are also predicted to house IgE binding epitopes (Lamontagne et al., 1996). Four

Table 1. Pollen allergens from various sources for which entire coding sequences have been cloned.

Source	Common name	Scientific name	Pollen allergen	References (see below)
Weeds	Short ragweed	<i>Ambrosia artemisiifolia</i>	Amb a 1, Amb a 2, Amb a 5, Amb a 6	1, 2, 3, 4
	Western ragweed	<i>Ambrosia psilostachya</i>	Amb p 5	5
	Giant ragweed	<i>Ambrosia trifida</i>	Amb t 5	6
		<i>Parietaria judaica</i>	Par j 1, Par j 2	7, 8
Grasses	Bermuda grass	<i>Cynodon dactylon</i>	Cyn d 1, Cyn d 7, Cyn d 12	9, 10, 11
	Orchard grass	<i>Dactylis glomerata</i>	Dac g 2, Dac g 3	12, 13
	Velvet grass	<i>Holcus lanatus</i>	Hol l 1, Hol l 5	14, 15
	Perennial ryegrass	<i>Lolium perenne</i>	Lol p 1, Lol p 2, Lol p 3, Lol p 5	16, 17, 18, 19, 20
		<i>Lolium italicum</i>	Lol i 2	63
	canary grass	<i>Phalaris aquatica</i>	Pha a 1, Pha a 5	21
	Timothy grass	<i>Phleum pratense</i>	Phl p 1, Phl p 2, Phl p 5, Phl p 6, Phl p 7, Phl p 11, Phl p 13	22, 23, 24, 25, 66, 26, 27
	Kentucky blue grass	<i>Poa pratensis</i>	Poa p 2, Poa p 9	63, 28
	Johnson grass	<i>Sorghum halepense</i>	Sor h 1	29
	Wheat	<i>Triticum aestivum</i>	Tri a 2	63
	Maize	<i>Zea mays</i>	Zea m 1, Zea m 11	58, 59,
	Barley	<i>Hordeum vulgare</i>	Hor v 9	56
	Rice	<i>Oryza sativa</i>	Ory s 1	57
Trees	Alder	<i>Alnus glutinosa</i>	Aln g 1, Aln g 4	30, 32
	White birch	<i>Betula verrucosa</i>	Bet v 1, Bet v 2, Bet v 3, Bet v 4, Bet v 5	32, 33, 34, 35, 36
	Hornbeam	<i>Carpinus betulus</i>	Car b 1	37
	Japanese cypress	<i>Chamaecyparis obtusa</i>	Cha o 1, Cha o 2	38, 39
	Hazel	<i>Corylus avellana</i>	Cor a 1	40
	Japanese cedar	<i>Cryptomeria japonica</i>	Cry j 1, Cry j 2	41, 42, 43, 44
		<i>Cupressus arizonica</i>	Cup a 1	68
		<i>Cupressus sempervirens</i>	Cup s 1	65
		<i>Juniperus ashei</i>	Jun a 1, Jun a 2, Jun a 3	46, 47, 67
		<i>Juniperus oxycedrus</i>	Jun o 2	45
	Eastern red cedar	<i>Juniperus virginiana</i>	Jun v 1, Jun v 3	64
	Privet	<i>Ligustrum vulgare</i>	Lig v 1	48
	Lilac	<i>Syringa vulgaris</i>	Syr v 1	49
	Annual dog's mercury	<i>Mercurialis annua</i>	Mer a 1	50
	Olive	<i>Olea europea</i>	Ole e 1, Ole e 2, Ole e 3, Ole e 6, Ole e 8	51, 52, 53, 54, 61
Field crops	Turnip	<i>Brassica rapa</i>	Bra r 1, Bra r 2	55, 62
	Oilseed rape	<i>Brassica napus</i>	Bra n 1, Bra n 2	62
	Sunflower	<i>Helianthus annuus</i>	Hel a 2	60

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regions in Hol 1 1 have been identified as housing IgE binding epitopes (Schramm et al., 1996). One of these regions is close to regions identified in Fes e 1 (Hiller et al., 1997) and Lol p 1 (Lamontagne et al., 1996) and therefore may represent a cross-reactive IgE epitope of the group 1 grass pollen allergens (Hiller et al., 1997). The T cell epitopes of two group 1 grass pollen allergens have been identified. For Phl p 1, 55% of T cell donors react to the immunodominant epitope spanning amino acids 70 to 84 (Schenk et al., 1995). This study also showed evidence that a degree of cross-reactivity may exist between T cell epitopes of group 1 grass pollen allergens from different species (Schenk et al., 1995). For Lol p 1, 3 immunodominant T cell epitopes have been predicted with one epitope (spanning amino acids 193 to 206) demonstrating immunodominance in three different studies (Perez et al., 1990; Spiegelberg et al., 1994; Bungy et al., 1994).

Cloning and sequencing of the group 1 grass pollen allergens has also allowed demonstration of structural similarities with functional proteins, and subsequent speculation about the biological role of the allergenic pollen proteins. The group 1 grass pollen allergens show approximately 25% amino acid homology α expansins, a group of proteins which promote plant cell wall enlargement (Shcherban et al., 1995). An expansin-like activity has even been demonstrated for maize pollen extract and its purified group 1 allergen Zea m 1, leading to the proposal that the pollen allergens be referred to as β expansins (Cosgrove et al., 1997).

Amino acid sequence homology has also been shown between the group 1 grass pollen allergens and three functionally important sequence motifs including the cysteine residue central to the active site of the C1 (papain-like) family of cysteine proteinases (Grobe et al., 1999). Recombinant Phl p 1 has also been shown to demonstrate proteinase activity, observed using a number of substrates, and which could be inhibited by various proteinase inhibitors (Grobe et al., 1999). Furthermore, Pike and colleagues have demonstrated immunological cross-reactivity between anti-Lol p 1 monoclonal antibodies and bromelain, a cysteine proteinase from *Ananas comosus* (pineapple) (Pike et al., 1997).

Groups 2 and 3. The groups 2 and 3 allergens of rye grass pollen are minor allergens, each is recognized by the sera of 45% of grass pollen allergic patients (Freidhoff et al., 1986). Lol p 2, an 11 kDa protein, is comprised of a group of acidic proteins with at

least two isoforms. Isoform A has been cloned and sequenced revealing a 97 amino acid residue protein with no cysteine residues and no glycosylation motif (Ansari et al., 1989b). Lol p 3, also an 11 kDa protein, is a group of basic proteins with at least two isoforms. Isoform B, the most abundant form, is the same size as Lol p 2 (97 amino acids) with which it shares 59% amino acid identity (Ansari et al., 1989a). Lol p 2 and 3 also share immunological cross-reactivity (Ansari et al., 1987). Sequences encoding the group 2 pollen allergens have also been isolated from Bermuda grass, wheat, velvet grass, Kentucky bluegrass and *Lolium italicum* (see Table 1). The group 3 allergen isolated from the pollen of orchard grass is a major allergen of this species, recognized by more than 60% of individuals allergic to pollen from this grass (Guerin-Marchand et al., 1996).

Group 5. The group 5 grass pollen allergens are non-glycosylated proteins of between 28–31 kDa (Singh et al., 1991) and are found exclusively in species belonging to the *Pooideae* subfamily (Smith et al., 1994). Group 5 pollen allergens have been cloned from five *Pooideae* species – velvet grass, rye grass, timothy grass, Kentucky bluegrass and canary grass (Table 1). These allergens share significant amino acid homology and immunological cross-reactivity.

Although Lol p 5 has been shown to consist of 8 isoforms (Smith et al., 1994), only three have been cloned (Singh et al., 1991; Ong et al., 1993; Suphioglu et al., 1999). The IgE binding epitopes of Lol p 5A and Lol p 5B have been mapped. For Lol p 5A, two strong IgE-binding epitopes were identified using overlapping synthetic peptides (Suphioglu et al., 1998). Significant amino acid homology exists between these regions of Lol p 5 and the corresponding regions of taxonomically related grasses, indicating that these regions may be important as cross-reactive IgE-binding epitopes. Two immunodominant IgE binding epitopes have also been identified for Lol p 5B (Ong et al., 1995). While one of these regions corresponds to one identified for Lol p 5A (Suphioglu, 1994), the other region was found to be unique to Lol p 5B. T cell epitopes of Lol p 5 and Phl p 5 have been predicted by various methods. Blaher and colleagues identified two immunodominant T cell epitopes for Lol p 5 while three immunodominant T cell epitopes were identified for Phl p 5 (Blaher et al., 1996; Muller et al., 1998). The existence of cross-reactive T-cell epitopes have been demonstrated between the group 5 allergens from timothy grass, rye

grass, Kentucky bluegrass and orchard grass (Muller et al., 1996).

Cloning and expression of Phl p 5 has allowed demonstration of an RNase activity for this recombinant allergen (Bufe et al., 1995). This observation, and the considerable amino acid homology between the group 5 grass pollen allergens, has led to the hypothesis that the group 5 grass pollen allergens may function as RNA degrading enzymes during pollen tube formation and elongation (Bufe et al., 1995).

Groups 6 and 7. Phl p 6 is a small 13 kDa protein which reportedly shows weak cross-reactivity to Phl p 5 (Petersen et al., 1995a/b). Phl p 7 and Cyn d 7 are minor allergens of timothy grass and Bermuda grass respectively since they are recognized by only 10% of pollen allergic patients. Both these allergens are calcium-binding proteins, and each possesses two EF-hand calcium-binding domains (Neiderberger et al., 1999). Cyn d 7 has been demonstrated to show significant sequence similarity to other calcium-binding allergenic proteins (Smith et al., 1997).

Groups 11, 12, 13. The group 11 allergens cloned from the pollen of timothy grass and maize have also been identified as profilins (Valenta et al., 1994; Heiss et al., 1996b). Recombinant Phl p 11, shown to possess up to 79% amino acid homology with other plant profilins, shares cross-reactive IgE epitopes with birch profilin (Valenta et al., 1994). Cyn d 12 is also a minor allergen of Bermuda grass pollen, being recognized by 20% of pollen allergic patients (Asturias et al., 1997a). Recombinant Cyn d 12, identified as a profilin, a member of a family of actin-binding proteins found in all eukaryotic organisms, has been shown to share IgE epitopes with profilins from the pollen of other plant species (Asturias et al., 1997a). A newly characterized, high molecular weight allergen has also recently been cloned from timothy grass. This allergen, designated Phl p 13, is approximately 55–60 kDa, shows homology with polygalacturonases and may be involved in food allergy in pollen sensitive patients (Suck et al., 2000).

Allergenic weed species

Seven genera of weeds have been reported to have clinical importance as sources of pollen allergenic proteins. *Ambrosia artemisiifolia* (short ragweed) is the major cause of late summer seasonal allergic rhinitis in eastern North America (King et al., 1964;

King, 1976). *A. psilostachya* (Western ragweed) and *A. trifida* (giant ragweed) are also known to possess allergenic pollen proteins. The pollen of *Artemisia vulgaris* (mugwort) is considered significant as a cause of late summer-autumn pollen sensitivity in many parts of the world. Allergenic proteins have been identified in the pollen of this species and also in the pollen of two other *Artemisia* species – *A. princeps* and *A. scoparia* (Park et al., 1989; Jaggi and Gangal, 1987). The allergens in mugwort pollen have also recently been implicated as a cause of food sensitivity due to at least one cross-reactive protein present in both mugwort pollen and certain food extracts (Caballero and Martin-Esteban, 1998). Mugwort pollen has also recently been shown to possess allergenic proteins cross-reactive with components from the pollen of ragweed, birch and timothy grass and therefore may contribute to the polysensitization of many sufferers (Heiss et al., 1996a; Grote et al., 1998; Hirschwehr et al., 1998).

The pollen of *Parietaria* species, which accounts for up to 42% of the annual pollen catch in Italy, is responsible for allergy in 40 to 50% of pollen sensitive adults in this region (Accorsi and Bandini Mazzanti, 1980; Geraci et al., 1981; D'Amato and Lobefalo, 1989). *Parthenium hysterophorus* (American feverfew) is a *Compositae* weed and a significant cause of pollen allergy in the Indian subcontinent, and certain parts of the southern United States and Australia (Wedner et al., 1989). A high degree of immunological cross-reactivity has been demonstrated between American feverfew and ragweed pollen extracts (Sriramarao and Rao, 1993). The pollen of *Echium plantagineum* (Paterson's curse), a problem weed of rural Australia, has been shown to induce an IgE response in atopic individuals (Katelaris et al., 1982). Although an insect-pollinated species, the pollen of Paterson's curse has been shown to reach the atmosphere in amounts sufficient to present a problem to sensitive individuals (Katelaris et al., 1982). *Plantago lanceolata* (plantain) has also been shown to induce indicators of type 1 allergic reactions in atopic individuals (Baldo et al., 1982). The pollen of *Mercurialis annua* (annual dog's mercury) has also been shown to cause allergic sensitization in atopic individuals in Spain (Garcia-Ortega et al., 1992).

Cloned weed pollen allergen genes

Four pollen allergens have been cloned from short ragweed – groups 1, 2, 5, and 6 allergens (Table 1). Amb a 1 has been identified as a major allergen

of short ragweed. Pollen allergens have also been cloned from two other *Ambrosia* species, Western ragweed and giant ragweed. Both group 5 allergens, these proteins show immunological cross-reactivity with the group 5 pollen allergen from short ragweed, Amb a 5 (Roebber et al., 1985; Ghosh et al., 1994) and with each other. Two allergens have been cloned from *P. judaica* (Ford et al., 1986) (Table 1). Par j 1, a 14 kDa molecular weight protein, is the major pollen allergen of this species being recognized by the sera of 95% of *P. judaica* allergic individuals (Costa et al., 1994). Par j 2 also a major allergen of this species is hypothesized to be the low molecular weight protein that shows cross-reactivity with proteins from other *Parietaria* species (Duro et al., 1996). The cloned *Parietaria* pollen allergens do not appear to share sequence homology or cross-reactive epitopes with pollen allergens from other genera. The cDNA encoding the panallergen profilin has been cloned from the pollen of annual dog's mercury (Vallverdu et al., 1998). Recombinant Mer a 1, produced in *E. coli*, was shown to share cross-reactive IgE epitopes with sunflower profilin (Vallverdu et al., 1998).

Allergenic tree species

Trees are large plants, commonly growing to over two meters, have woody stems and are long lasting, but slow growing. In temperate climates such as parts of Europe, pollen from the trees of the *Fagales* order not only predominate the atmosphere (Eriksson, 1991) but they are the major source of springtime allergies (Charpin et al., 1974; Eriksson, 1978). Birch pollen alone is estimated to be responsible for 22% of the major European seasonal allergic diseases (Jarolim et al., 1989). A number of other tree species including *Chamaecyparis obtusa* (Japanese cypress), *Cryptomeria japonica* (Japanese cedar), *Olea europaea* (olive), *Fraxinus excelsior* (ash), *Juniperus oxycedrus*, *Ligustrum vulgare* (privet), and *Syringa vulgaris* (lilac) are important contributors to pollen allergy in various parts of the world. In Japan, the pollen of Japanese cedar is the most common cause of immediate hypersensitivity, affecting between 25 and 40% of allergic individuals (Ishizaki et al., 1987; Griffith et al., 1993). In the Mediterranean region, trees of the *Oleaceae* family, including olive, ash, privet and lilac, are important sources of aeroallergens with the pollen of the olive tree being responsible for allergy in 30 to 40% of all pollen allergic individuals in Bari, Italy (Macchia et al., 1991). Extensive

immunological cross-reactivity has been demonstrated between the pollen extracts of four *Oleaceae* species, olive, ash, privet and lilac (Bousquet et al., 1985b). Consequently, sensitivity to the pollen of one species may preclude development of sensitivity to all four species in susceptible individuals.

Sensitivity to the pollen of *Acacia* species was first documented in 1981 (Geller and Rosario, 1981). This study revealed that only 11% of asthmatics and/or rhinitic patients showed sensitivity to *Acacia* pollen in skin prick tests. In the Mediterranean area, one study has demonstrated that only 1.2% of a population of atopics, but 31% of patients who are occupationally exposed show sensitivity to the pollen of *A. floribunda*, demonstrating its importance as a source of occupational allergy (Ariano et al., 1991). Two further studies have shown the level of allergic sensitization to *Acacia* pollen in two atopic populations in the Asia-Pacific region – in an Indonesian population, over 12% of atopic patients showed positive skin prick tests, while in a Malaysian population, more than 20% were positive (Sam et al., 1998; Baratawidjaja et al., 1999). The pollen of *Pyrus pyrifolia* (Japanese pear) has also recently been shown to be a source of aeroallergens. Being an entomophilous species, allergy to the pollen of Japanese pear has only been demonstrated in farmers working on artificial pollination of this species (Yanagisawa et al., 1999).

Cloned tree pollen allergen genes

The first pollen allergen gene to be cloned was the major birch allergen Bet v 1 (Breiteneder et al., 1989). This protein binds IgE from over 90% of birch pollen sensitive patients (Breiteneder et al., 1989). Bet v 1 has since been shown to be represented by 13 isoforms (Swoboda et al., 1995) which vary in IgE reactivity (Ferreira et al., 1996). Native and recombinant Bet v 1 have also been shown to possess RNase activity and may thus function as RNA degrading enzymes upon release from pollen during fertilization (Bufe et al., 1996). Group 1 allergens cloned from the pollen of other *Fagales* trees, including *Corylus avellana* (hazel), *Alnus glutinosa* (alder) and *Carpinus betulus* (hornbeam), show cross-reactivity with each other and with Bet v 1. Bet v 2, a 14 kDa molecular weight protein has been identified as a profilin, and shows cross-reactivity with other cloned profilin allergens from fruits and vegetables (Ebner et al., 1995; Bauer et al., 1996). Bet v 1 and Bet v 2, which appear to be the most allergenic pollen proteins from these trees, together harbor most of the IgE epitopes of the

Fagales pollen allergens (Neiderberger et al., 1998). Bet v 3 and Bet v 4 have been identified as calcium-binding allergens, with Bet v 4 showing immunological cross-reactivity to other calcium-binding allergenic proteins, including Cyn d 7 from Bermuda grass (Seiberler et al., 1994; Engel et al., 1997; Smith et al., 1997). The recent cloning and characterization of Bet v 5, a minor allergen from the pollen of birch, has identified this protein as belonging to a family of isoflavone reductase-related proteins involved in plant defense reactions (Karamloo et al., 1999). It is predicted that Bet v 5, a 33 kDa molecular weight allergen may also partly contribute to food-sensitivity in birch pollen sensitive individuals since it has been shown to possess IgE cross-reactivity with comparable molecular weight proteins in a number of edible fruits (Karamloo et al., 1999).

Group 1 allergens have also been cloned from the pollen of members of the *Oleaceae* family – olive, ash, privet and lilac. These allergens also share immunological cross-reactivity with each other (Bousquet et al., 1985b; Kernerman et al., 1992; Obispo et al., 1993). Two immunodominant T cell epitopes of the major allergen from olive pollen, Ole e 1, have been identified (Carbada et al., 1998). Recently four other allergens have been cloned from the pollen of the olive tree including olive profilin, Ole e 2 (Asturias et al., 1997b). The cloning of Ole e 3 allowed its identification as a calcium-binding major allergen from olive pollen (Ledesma et al., 1998). Cross-reactive homologues of Ole e 3 have been detected in protein extracts from a variety of olive-tree and non-related mature pollens (Ledesma et al., 1998). The cloning and sequencing of Ole e 6, showed that this allergen does not appear to share sequence similarity with any other previously described polypeptide (Batanero et al., 1997). Ole e 8 is also a calcium-binding allergen from the pollen of the olive tree (Ledesma et al., 2000). Group 1 allergens cloned from Japanese cedar (Cry j 1) and Japanese cypress (Cha o 1) have recently been shown to share common IgE binding epitopes (Kinettsu et al., 2000) and one immunodominant T cell epitope (Ohno et al., 2000). These allergens along with recombinant Jun a 1 (Midoro-Horiuti et al., 1999) have been characterized as pectate lyases (Mari, 2000). Sequences encoding the group 1 pollen allergens from *Juniperus virginiana* (Eastern red cedar), *Cupressus sempervirens* and *C. arizonica* have also recently been isolated (Midoro-Horiuti et al., 1999; Aceituno et al., 2000; Monsalve et al., 2000). The group 2 allergens Cry j 2 and Cha o 2 have been iden-

tified as polygalacturonases (Mari, 2000). The group 2 allergen from the pollen of *J. ashei* (Mountain cedar) has also been cloned and shown to possess considerable amino acid sequence homology with Cry j 2 and Cha o 2 (Yokoyama et al., 2000). Recombinant Jun a 3 has been identified as a pathogenesis-related protein, and Jun o 2 as a calcium-binding allergenic protein (Mari, 2000).

Allergenic crop and ornamental plant species

Reports of allergy resulting from exposure to the pollen of crop and ornamental plant species are on the increase (Blanco et al., 1998; Chowdhury et al., 1998; Tanaka et al., 1998; Ortega et al., 1999). Due to the nature of crop and ornamental plant farming, where plants are usually grown at high density in vast fields, but are typically grown in sparsely populated areas, allergy to the pollen of crop and ornamental species typically develops as a result of occupational exposure.

Brassica napus (oilseed rape), a largely insect-pollinated crop, is cultivated widely around the world primarily as a source of oil. Allergy to oilseed rape pollen was initially reported over 40 years ago (Colldahl, 1954). A number of volatile organic compounds released by oilseed rape during flowering have also been identified as potential aeroallergens/irritants (Butcher et al., 1994). However, a recent study showed that 7.1% of a population of pollen-allergic patients were sensitive to oilseed rape pollen in skin prick tests, indicating that oilseed rape pollen is also a relevant source of pollen allergens (Hemmer et al., 1997). Furthermore, allergens from the pollen of oilseed rape have been found to demonstrate immunological cross-reactivity to pollen proteins from rye grass, alder and birch (Focke et al., 1998). Allergenic proteins have also been identified from the pollen of turnip and *B. campestris* (Canola) (Toriyama et al., 1995; Singh et al., 1995).

Due to its entomophilous nature, allergy to the pollen of sunflower was initially believed to be the result of occupational exposure, and was even demonstrated in one human experimental model (Bousquet et al., 1985a). But more recently, atopic individuals living in sunflower-growing areas, but not occupationally exposed, were found to be sensitive to sunflower pollen (Jimenez et al., 1994). Cross-reactivity between the pollen of sunflower and that of other *Compositae* plant species (mugwort, short ragweed, *Leucanthemum vulgare* (marguerite), *Taraxacum officinale*

(dandelion) and *Solidago* sp. (golden rod) has also been demonstrated with mugwort exhibiting the highest level of cross-reactivity (Fernandez et al., 1993). Four species of the *Pistachia* genus (Pistachio nut) have also been shown to possess allergenic pollen (Keynan et al., 1987). These pollens have been shown to exhibit allergenic cross-reactivity with each other and also the closely related *Schinus terebintifolius* (Keynan et al., 1997). Allergenic proteins have also been detected in the pollen of *Cannabis sativa* (marijuana), *Ipomoea fistulosa* (sweet potato) and the ornamental crop species *Humulus japonicus* (Japanese hop) (Mondal et al., 1998; Tanaka et al., 1998; Park et al., 1999).

Cloned crop and ornamental plant pollen

Allergen Genes

Allergens have been cloned from the pollen of a number of field crop plant species including turnip, oilseed rape and sunflower (Table 1). Bra r 1 from turnip possesses two calcium-binding domains and has been shown to bind calcium (Toriyama et al., 1995). IgE binding to Bra r 1 has recently been shown to be dependent on its ability to bind calcium (Okada et al., 1998). This protein shares a significant level of amino acid similarity with other cloned calcium-binding allergens, including Cyn d 7 and Bet v 4, and has been shown to possess immunological cross-reactivity with Cyn d 7 (Smith et al., 1997). The gene encoding Hel a 2, a minor pollen allergen from sunflower, has been cloned and identified as the actin-binding protein, profilin (Asturias et al., 1998). Recombinant Hel a 2 shows cross-reactivity with profilins from other *Compositae* species including mugwort and short ragweed (Fernandez et al., 1993) and taxonomically distinct plants such as Bermuda grass, the olive tree and annual dog's mercury (Asturias et al., 1998).

Cross-reactive allergens and associated allergies

It has recently been shown that allergens from different sources can show immunological cross-reactivity, often resulting from conservation of IgE epitope regions. Allergens with similar biological properties may also demonstrate a degree of immunological cross-reactivity. A class of calcium-binding allergens identified from the pollens of Bermuda grass, birch, sunflower and oilseed rape represents such a group of cross-reactive proteins. Within the defined calcium-binding domains of these proteins there is

significant amino acid conservation. For the allergens from birch and oilseed rape, IgE reactivity has been shown to be dependent on the ability of the proteins to bind calcium ions (Seiberler et al., 1994; Okada et al., 1998). The birch allergen has been demonstrated to be highly cross-reactive with proteins from the pollen of timothy grass, mugwort and lily (Engel et al., 1997).

Pollen allergic patients often display allergic reactions to pollen and food derived from distantly related plant species to the sensitizing allergen(s). Birch pollen sensitive individuals often display sensitivity to foods such as apple, kiwi fruit, carrot, potato and hazelnut. Ragweed pollinosis often leads to sensitization to watermelon, melon, cucumber and banana while mugwort pollinosis is often associated with hypersensitivity to celery, carrot, nuts, spices, mustard and leguminous vegetables. Grass-pollen sensitive individuals often react with tomato, potato, green pea, peanut, watermelon, melon, apple, orange, kiwi (for review see Caballero and Martin-Esteban, 1998). Many studies have demonstrated that these pollen and food extracts contain proteins cross-reactive with known allergens and which therefore may contribute to food sensitivity in pollen sensitive individuals (Tables 2 and 3).

At least four distinct cross-reactive proteins have been identified in pollen and food extracts, corresponding to 14, 16 to 17, 35, and 60–69 kDa proteins. The 14 kDa proteins represent profilins, a family of actin-binding proteins found in all eukaryotic organisms, and predominantly the most widespread group of cross-reactive allergens. Not identified in plants until the isolation of Bet v 2 from birch pollen (Valenta et al., 1991), profilins have subsequently been identified in, and isolated from, the pollen of many other plant species (see Table 3). These proteins are known to induce allergic responses in sensitive individuals and are cross-reactive with each other. Since profilins are present in all eukaryotic organisms, primary sensitization to an allergenic pollen profilin may lead to secondary sensitization to profilins present in food extracts (Table 3). Furthermore, profilin has been identified as an IgE-binding component of latex extracts (Vallier et al., 1995) and therefore may also contribute to sensitization to latex extracts in pollen allergic individuals.

A major allergen of birch pollen, Bet v 1, a 16–17 kDa protein is not only cross-reactive with known allergens from the pollen of other tree species, but also with proteins from apple, pear, sweet cherry, celery and carrot extracts (see Table 2). Bet v 1 cross-

Table 2. Cloned allergens (excluding profilins), food and pollen extracts that possess proteins cross-reactive with known pollen allergens and which may contribute to food sensitivity in pollen allergic individuals.

Protein size	Cloned allergens	Food/pollen extracts	References
16–17 kDa	Bet v 1 (birch)		15
	Mal d 1 (apple)		13
	Pru a 1 (sweet cherry)		16
	Api g 1 (celery)		1, 2, 3
	Dau c 1 (carrot)		12
		Anise	4
		Fennel	4
		Coriander	4
		Cumin	4
		Mugwort pollen	5
		Celery	5, 6
		Apple	6
		Poppy	11
		Pear	6
35 kDa		Lychee	14
		Mango	14
		Banana	14
		Orange	14
		Apple	14
		Pear	14
60–69 kDa		Carrot	14
		Mugwort pollen	8
		Birch pollen	7, 8, 9
		Timothy grass pollen	7, 8
		Apple	7, 8
		Kiwi fruit	8
		Celery	5, 7, 8, 9
		Pepper	9
		Anise	4
		Fennel	4
		Coriander	4
		Cumin	4
		Peanuts	8

References: 1. Scheurer et al., 1999. 2. Breiteneder et al., 1995. 3. Hoffman-Sommergruber et al., 1999a. 4. Jensen-Jarolim et al., 1997. 5. Bauer et al., 1996. 6. Ebner et al., 1995. 7. Grote et al., 1998. 8. Heiss et al., 1996a. 9. Leitner et al., 1998. 10. Scheurer et al., 2000. 11. Jensen-Jarolim et al., 1999. 12. Hoffman-Sommergruber et al., 1999b. 13. Hoffman-Sommergruber et al., 1996. 14. Wellhausen et al., 1996. 15. Breiteneder et al., 1989. 16. Scheurer et al., 1997.

reactive homologues of comparable molecular weight have also been detected in preparations of spices such as coriander, cumin, anise and fennel (see Table 2).

A 35 kDa allergenic protein has been identified in the pollen of birch (Wellhausen et al., 1996). This protein, which represents a minor allergen of birch pollen, demonstrates cross-reactivity with proteins of comparable molecular weight in extracts of carrot and a number of fruits. The 60–69 kDa cross-reactive component of mugwort pollen is Art v 1, a major

Table 3. Allergenic profilins cloned or detected in pollen or food extracts.

	Allergen	Origin	References
Cloned allergens	Bet v 2	Birch pollen	1
	Cyn d 12	Bermuda grass pollen	2
	Mer a 1	<i>Mercurialis annua</i> pollen	3
	Phl p 11	Timothy grass pollen	4
	Hel a 2	Sunflower pollen	5
	Ole e 2	Olive tree pollen	6
	Zea m 11	Maize pollen	7
	Gly m 3	Soybean	19
	Pyr c 4	Pear	25
	Pru av 4	Cherry	25
	Api g 4	Celery	26
		Castor bean	27
		<i>Arabidopsis thaliana</i>	28
		Barley	28
		Peanut	20
		Bean	21
		Tobacco	14
		Wheat	16
		Tomato	8
Detected in pollen/food extracts		Oilseed rape pollen	9
		Lily pollen	10
		Celery	11
		Mugwort pollen	12
		Poppy seeds	13
		Johnson grass pollen	11
		Kentucky bluegrass pollen	11
		Apple	11
		Carrot	11
		Potato	15
		European chestnut	17
		Bell peppers	22
		Poppy pollen	23
		Ragweed pollen	12
		Lychees	24
		Rye grass pollen	18

References: 1. Valenta et al., 1991. 2. Asturias et al., 1997a. 3. Vallverdu et al., 1998. 4. Valenta et al., 1994. 5. Asturias et al., 1998. 6. Asturias et al., 1997b. 7. Staiger et al., 1993. 8. Yu et al., 1998. 9. Focke et al., 1998. 10. Vidali et al., 1999. 11. Vallier et al., 1992. 12. Hirschwehr et al., 1998. 13. Jensen-Jarolim et al., 1999. 14. Mittermann et al., 1995. 15. Ebner et al., 1995. 16. Rihs et al., 1994. 17. Hirschwehr et al., 1993. 18. van Ree et al., 1992b. 19. Rihs et al., 1999. 20. Kleber-Janke et al., 1999. 21. Vidali et al., 1995. 22. Jensen-Jarolim et al., 1998. 23. Clarke et al., 1998. 24. Fah et al., 1995. 25. Scheurer et al., 2001. 26. Scheurer et al., 2000. 27. Schobert et al., 2000. 28. Unpublished.

allergen of this species. Food extracts to which mugwort pollen sensitive individuals react is often due to the presence of cross-reactive proteins of comparable molecular weight to Art v 1 (Heiss et al., 1996a). Cross-reactive proteins have been identified in certain tree, grass, spice, fruit and vegetable species (see Table 2).

Movement of pollen allergens through the environment

Pollen and pollen allergens move through the atmosphere via a number of methods. The action of wind and/or insects can naturally aid transmission of whole pollen grains upon anther dehiscence. The risk of sensitization to wind pollinated (anemophilous) flowering plant species is high since these species produce copious amounts of pollen that may be carried in the atmosphere for considerable distances (Smart et al., 1979). On the other hand, insect pollinated (entomophilous) species produce pollen that is adhesive making grains suited to sticking to the bodies of insects (Jimenez et al., 1994). Unassisted, the pollen of these species travel only short distances in the atmosphere, so the risk of sensitization is often limited to people who are in close contact with such species (Solomon, 1984). For example, ryegrass is an anemophilous species, producing up to 464 kg of pollen per hectare of an unmown, ungrazed field (Smart and Knox, 1979). In many parts of the world, the pollen of ryegrass is responsible for pollen allergy in more individuals than any other flowering plant species. In comparison, sunflower is an entomophilous species – sunflower pollen-induced allergy is typically restricted to occupationally exposed individuals, and those frequenting the growing regions (Jimenez et al., 1994).

Interaction between pollen aeroallergens and air pollution

Environmental pollutants such as from industrial emissions, diesel exhaust particulates (DEP), and gaseous pollution have been found to be associated with allergic sensitization and disease (Behrendt et al., 1995). There is evidence to suggest that air pollutants, such as those from industrial and vehicular emissions, interact with the pollen of flowering plants causing alteration of pollen grain morphology, affecting protein and allergen release, and influencing the generation of allergen-containing aerosol particles (Behrendt et al., 1997). Significant alterations to pollen grain morphology, both at the surface and within the pollen protoplasm, have been demonstrated upon the interaction of pollen grains with particulate and gaseous air pollutants (Behrendt et al., 1997). These changes to pollen grain structure and morphology have been shown to promote protein release from pollen grains, in a dose- and time-dependent

manner (Behrendt et al., 1991). The native form of the major allergen from rye grass pollen, Lol p 1, has been shown to bind to respirable DEP present in air pollution (Knox et al., 1997). In this form, pollen allergens may become concentrated in the particulate matter and thereby present a greater risk of exposure upon encounter by sensitized individuals. Gaseous air pollutants, in particular sulfur dioxide, have also been shown to interact with pollen grains. Highly concentrated sulfur dioxide has been demonstrated to significantly reduce the release of the major pollen allergen from timothy grass, thereby reducing bioavailability of the allergen to respirable particles (Behrendt et al., 1997). Particulate and gaseous air pollutants may also play a role in the uptake of allergen molecules into the human circulatory system, since it is hypothesized that DEP, sulfur dioxide and nitrogen oxides increase mucosal permeability, thereby enhancing allergen movement through the mucosa (Roitt et al., 1998). Furthermore, DEP have experimentally been demonstrated to act as powerful adjuvants promoting IgE production when co-administered intranasally with the sensitizing agent (Takafuji et al., 1987).

Effect of paucimicronic particles on environmental allergen transmission

Allergic manifestations such as bronchial asthma and respiratory allergy typically result from allergen penetration into the medium and small airways. Due to their size, whole airborne pollen grains usually become trapped in the upper respiratory tract or the mucosal surfaces of the nose and throat, and rarely penetrate into the smaller airways (Wilson et al., 1973; Rosenberg et al., 1983). Allergen-carrying, paucimicronic particles, including starch granules released from pollen under wet conditions or during thunderstorms, are smaller than whole pollen and are therefore able to penetrate deep into the airways. Studies on the pollen allergens from *Parietaria judiaca*, ragweed, and rye grass have demonstrated the presence of allergens in atmospheric particles smaller in size than the pollen from which they are derived (Busse et al., 1972; Solomon et al., 1983; D'Amato et al., 1994; Spieksma et al., 1995). These particles are not only small enough to penetrate the lower airways on their own, but they may also be transferred by association with respirable particulate matter present in the atmosphere, such as DEP (D'Amato, 2000).

Conclusions

Allergy to pollen-derived proteins from many common flowering plant species impacts on the lives of many people. An otherwise innocuous component of the environment we live in, pollen is one of the most predominant sources of environmental allergens. With the development of molecular biology techniques, the characterization and cloning of many allergenic pollen proteins and their encoding genes have been completed. This application of recombinant DNA technology has taken the study of pollen allergens and the nature of the allergic response to new levels of understanding. It has allowed characterization of protein structure and thus demonstration of structural and immunological similarities of allergens with each other and with functional proteins. This factor has also helped explain allergenic cross-reactivity between allergens from both closely and distantly related plant species. A number of cloned and characterized pollen allergens have also been useful in demonstrating the presence of cross-reactive proteins in food extracts, and thus providing an explanation for the phenomena of food sensitivity exhibited by many pollen sensitive individuals.

Difficult and costly to prepare by conventional protein purification methods, the cloning of pollen allergen genes has also permitted purification of recombinant allergen preparations, free from contaminating eukaryotic proteins, useful for diagnosis of allergen-specific sensitivity. Furthermore, the determination of the allergenic and immunologic components of cloned and characterized allergens have been made possible using recombinant DNA techniques. This information is vital in understanding how these proteins interact with the human immune system and how they may be modified for the development of allergen-specific immunotherapeutic reagents.

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