

Review article

Aeroallergens of plant origin: molecular basis and aerobiological significance

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Abstract

This review presents an update on the sources and molecular basis of aeroallergens of plants, derived from pollen, seeds, leaf and stem detritus and their protein molecules. These aeroallergens are a natural component of the atmosphere, either because of their natural function or human activity. Pollen is a source of allergens within the 10–200 μm size range, and while most allergenic pollen types account for only 20–30% of total annual pollen catch, during their flowering season, they are usually the dominant type. Tree pollen commences the season in winter, with birch pollen counts in Scandinavia being the highest daily pollen counts yet reported and a major allergen, a 14-kDa protein, which is similar to pathogenesis-related proteins. Grass pollen follows in spring, and is unique as its two immunodominant allergens, a 35-kDa glycoprotein and 28–32-kDa protein, are in different cellular sites: the cytosol and surface of pollen grains; and in intracellular starch granules. The allergens at the pollen surface are not inhalable and can interact only with the eyes, nasal and oral cavities. Starch granules are released to the atmospheric aerosol when grains rupture in rainwater. These are a major source of allergen-containing micronic particles, which are important because they are inhalable. At the same time, allergen molecules are present in the aerosol, and these can bind to soot particles, and so be respired deep into the airways. The major Japanese cedar pollen allergen has been detected both within the pollen and in orbicules; particles less than 1 μm that line the anther cavity and can be released into the air when dehiscence occurs. Ragweed is the major cause of late summer hayfever in eastern North America, where its pollen accounts for up to 41% of the annual pollen catch. It is a major source of aeroallergens in both respirable and non-respirable size ranges. As a result of human activity, dusts derived from seeds and cereal grains during transport, storage and milling provide a source of micronic particles, containing potent allergens that can trigger allergic disease.

Keywords: Aeroallergens; Pollen; Ragweed; Aerobiological significance

1. Introduction

Humankind co-exists in the global environment with plants. Plants produce propagules, pollen, seeds and chemicals, some of which are a natural part of the atmosphere. Humans come in contact with or inhale these atmospheric particles, which are part of the air they breathe. These particles may carry plant-derived proteins, glycoproteins or polysaccharides, which may

act as antigens in interacting with the human immune system. Of special interest are those antigens which act as allergens in eliciting the immediate hypersensitivity response. This response is mediated by the special atopic antibody, Immunoglobulin E (IgE, Druce, 1993). Exposure to allergens leads to: (1) production of specific IgE in the immune system; (2) circulation and binding of IgE to the surface of most cells and basophils; (3) binding of allergen molecules to adjacent pairs of IgE molecules; and (4) release of mediators of the allergic response. This can trigger allergic rhinitis (hayfever, symptoms — running nose and eyes), allergic asthma (symptoms —

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wheezing and shortness of breath), and atopic dermatitis (symptoms — inflammation of the skin) (Roitt et al., 1985). Allergens are commonly proteins or glycoproteins. A major allergen can induce an allergic reaction in more than 90% of patients allergic to the protein (Aas, 1978). A minor allergen is only important for a small number of patients (typically 20–30%). Allergens are named according to international convention, since in most cases, their natural biochemical function has seldom been established. The convention is that the allergen is named according to the name of the source genus (first three letters) and species (first letter) followed by a number, for example, the allergens of rye-grass, *Lolium perenne*, are named Lol p 1, Lol p 2, Lol p 3, etc. (King et al., 1994).

Allergen molecules are present in indoor and outdoor environments in many forms. Aeroallergens are present as a natural part of the atmosphere, where they occur in the form of dust, aerosols, cellular material, particles as small as 0.01 μm , and can be deposited on the mucous membranes of the eyes, nasal and oral cavity and airways. Aeroallergens include reproductive structures of many seed plants (pollen grains) and fungi (spores), as well as certain algae, bacteria, actinomycetes and protozoa, which regularly undergo atmospheric transportation. In addition, biogenic debris, including mammalian danders and arthropod emanations, are also aeroallergens (Solomon and Mathews, 1988).

In this review, we will focus on aeroallergens of plant origin.

2. Biodiversity of plant aeroallergens

Aeroallergens of plants can be derived from pollen of conifers and flowering plants, seeds, leaf and stem detritus and protein molecules, especially proteolytic enzymes. These allergens are a natural component of the atmosphere either through their biological activity (for example, pollen for fertilization) or human activity.

2.1. Pollen allergens

Pollen grains, which range in size from 3.5 μm to more than 200 μm , are dispersed into the air to effect fertilization and hence seed set. During these processes, pollen may contact the eyes, nose and upper respiratory tract of sensitive humans, eliciting symptoms of hayfever (Bush, 1989). The probability that allergic problems may occur is high with wind pollinated flowering plants (anemophilous), which produce high levels of aeroallergens. Entomophilous (insect pollinated) plants produce lower numbers of pollen grains, which are more sticky and travel shorter distances in the atmosphere (Solomon, 1984).

Pollen grains contain many different types of proteins, which are located in three major protein domains: the outer exine wall made of sporopollenin; the inner

pectocellulosic wall, the intine; and the cytoplasm (see Knox, 1984a,b). Not all proteins act as allergens. Allergen molecules are generally restricted in size to the mol. wt of common enzymes, that is, 10–40 kDa (Aas, 1978; Bush, 1989), and are released rapidly from pollen grains soon after moistening (Knox and Heslop-Harrison, 1971). The rate of release of allergens from the grain upon contact with mucous membranes is rapid, so that interaction occurs before mucociliary action can remove pollen grains to the glottis (within 6–8 min), where pollen may be swallowed and digested (Bridger and Proctor, 1971). The nasopharynx is the site of maximum exposure to rapidly released allergens (Baraniuk et al., 1988).

Pollen allergens are mobile molecules, diffusing from the grains within minutes of moistening (Knox and Heslop-Harrison, 1971; Taylor et al., 1994). For example, the major allergen of grasses and tree pollen are released within 10–16 min (Marsh et al., 1981; Vrtala et al., 1993). A minor birch allergen, profilin, accounted for IgE binding in 10% of birch pollen-allergic patients, but its release from the grain did not occur until after 10 min from moistening and in much smaller quantities than the major allergen. Allergens from short ragweed pollen also showed rapid release within 20 min of hydration (Marsh et al. 1981; Baraniuk et al. 1988), although a minor allergen, Amb a 5, (accounting for IgE binding in 13% of allergic patients) was completely released within 4 min. The release of the major allergen, Amb a 1, from the grains was much less rapid despite its higher allergenic potential (Aas, 1978). While allergenic proteins are released rapidly upon hydration, non-allergenic proteins, such as heat shock protein 70, are detected only after using harsh extraction methods, such as boiling in SDS sample buffer and grinding (Vrtala et al., 1993). Thus, most allergenic molecules are present in large amounts and show rapid times of release from pollen.

Tree aeroallergens: Trees are the earliest to undergo flowering in each season, starting in winter. Pollen grains from trees of the order Fagales (alder, birch, hazel, hornbeam and oak) are the major sources of springtime allergies in temperate climate zones (Charpin et al., 1974; Eriksson, 1978). In Europe, birch pollen is responsible for 22% of the major seasonal allergic diseases (Jarolim et al., 1989a). In Sweden, there is six times more birch pollen than grass pollen in the air (Eriksson, 1991). Birch pollen counts have been reported exceeding 3000 grains $\text{m}^{-3} \text{day}^{-1}$ (Vik et al., 1991). The major pollen allergen of birch (*Betula verrucosa*) is Bet v 1 (Ipsen and Løwenstein, 1983), where 56 out of 58 patients with birch pollen allergy possessed IgE to Bet v 1 (Jarolim et al., 1989b). It has been shown that many of the extractable birch pollen proteins have immunochemically similar counterparts in the pollen of other representatives of the family Betulaceae, including

alder, hazel and hornbeam. Clinical symptoms, resulting from exposure to pollens other than birch, often occur among patients with birch hayfever (Eriksson et al., 1987). This suggests that the major allergenic activity is confined to the cross-reactive Group I allergens, which appear to be similar for all members of the birch family. This observation is supported by comparisons of the amino acid sequences of these allergens which reveals a high degree of sequence identity (Ipsen and Hansen, 1990, 1991). Bet v 1 shows sequence homology with the pathogenesis-related proteins of other plants that are defence molecules against pathogens (Breiteneder et al., 1989). An interesting observation concerning Bet v 1 is the apparent effect of pollution as the allergen increases dramatically in pollen from trees growing in heavy traffic areas (Jilek et al., 1993).

A number of other tree species, particularly Japanese cedar, Oleaceae (ash, olive, privet) and cypress (Cupressaceae) are important allergen sources in several parts of the world. Pollen of Oleaceae (ash, olive, privet) is important in countries in the Mediterranean region. The incidence of hypersensitivity to olive pollen in Bari, Italy is 30–40% of all pollen-sensitive patients (Macchia et al., 1991). The major allergen of olive pollen, Ole e 1, consist of two subunits of 17 and 19 kDa. Eighty-seven percent of patients sensitive to olive pollen, as judged by case history and skin test, have IgE recognizing these proteins (Wheeler, 1992). Sensitization to pollen of olive can lead to allergic reactions from pollen of ash and privet. There is a high degree of allergenic cross-reactivity among the three species of Oleaceae (Bousquet et al., 1985; Kernerman et al., 1992; Obispo et al., 1993). Meanwhile, Japanese cedar (*Cryptomeria japonica*) is the most significant cause of seasonal rhinitis in Japan, affecting between 25 and 40% of the allergic population (Griffith et al., 1993). During spring, cedar pollen is the dominant pollen in the air (Ishizaki et al., 1987).

Grass aeroallergens: The widespread distribution of the grasses has made these the most important contributors to pollen-induced allergy. The majority of grass species of known allergic importance are representatives of the subfamily Pooideae, which includes orchard grass (*Dactylis glomerata*), timothy (*Phleum pratense*), meadow fescue (*Festuca elatior*), rye-grass (*Lolium perenne*) and Kentucky bluegrass (*Poa pratensis*), all cool-temperate grasses. The warm-temperate grasses of clinical significance are represented by Bermuda grass (*Cynodon dactylon*) in subfamily Chloridoideae, and the sub-tropical grasses are represented by Bahia grass (*Paspalum notatum*) and Johnson grass (*Sorghum halepense*), which belong to the subfamily Panicoideae.

The best characterized of those grasses include Bermuda grass, canary grass, cocksfoot, Kentucky bluegrass, perennial rye-grass, sweet vernal grass,

timothy and Yorkshire fog. The most comprehensive studies have been made of proteins from rye-grass pollen, and to a lesser extent, Kentucky bluegrass and timothy.

In the cool temperate climate of southern Australia, rye-grass pollen is present in greater abundance than other types of grass pollen (Smart et al., 1979). This is a consequence of the phenomenal productivity of rye-grass, with pollen yields reaching up to 464 kg per hectare in unmown and ungrazed rye-grass. In the cool temperate northern climates of California and Europe, rye-grass pollen is the major source of seasonal allergy (Spieksma, 1990), however, in other areas with similar climates, it is replaced in importance by pollen from closely related grass genera, *Poa* and *Phleum*. In North America, Kentucky bluegrass (*Poa pratensis*) and in Europe, timothy grass (*Phleum pratense*) are major pollen allergens.

The seasonal total of grass pollen counts varied markedly from one season to the next (Smart and Knox, 1979; Spieksma et al., 1985; Weeke and Spieksma, 1991). For example, the seasonal total grass pollen counts in Cardiff ranged from about 4800 in 1982 to 10 800 in 1986; in Brussels from about 2500 in 1986 to 7500 in 1983 (Weeke and Spieksma, 1991); in Leiden from 3834 to 5816 (Spieksma et al., 1985; Spieksma, 1986); and in Madrid from 2568 to 6624 (Subiza et al., 1992). In Melbourne, Australia, the seasonal grass pollen counts ranged from 2319 in 1976 to 8217 in 1993. The levels of grass pollen counts in different cities fall within the same range, the proportion of grass pollen compared to total annual pollen varies from 12 to 27% (Bousquet et al., 1984; Spieksma et al., 1985; D'Amato and Lobefalo, 1989; Ong et al., 1995).

The allergenic spectrum of rye-grass pollen is known to be complex (Ford and Baldo, 1986); the international reference preparation for rye-grass contains more than 17 allergens ranging in mol. wt from 12 to 89 kDa (Stewart et al., 1988). The major allergens of rye-grass pollen are Lol p 1, a 35-kDa glycoprotein (Johnson and Marsh, 1966; Howlett and Clarke, 1981; Cottam et al., 1986) and Lol p 5, which has been identified with a mol. wt ranging from 28 to 32 kDa (Singh et al., 1991; Ong et al., 1993).

Lol p 1 appears to be the major allergen responsible for sensitivity to rye-grass pollen since approximately 90% of rye-grass allergic patients demonstrate skin test reactivity to purified Lol p 1 (Lichtenstein et al., 1969; Freidhoff et al., 1986).

Lol p 5 is equivalent in importance to Lol p 1, as judged by the criteria of number of allergic patients responding to it (more than 90%) and strong IgE binding to the allergen. However, IgE binding appeared to be stronger to Lol p 5 than to the more abundant allergen Lol p 1 (Matthiesen and Løwenstein, 1991). Suphioglu (1994) found that Lol p 5 accounted for about 65% of the IgE

reactivity of the crude pollen extract. Together, Lol p 1 and Lol p 5 account for nearly all the IgE binding reactivity of crude rye-grass pollen extract (Bond et al., 1993).

The amino acid sequence of Lol p 5 shows no homology with Lol p 1, but significant homology with an allergen cloned from Kentucky bluegrass pollen (Silvanovich et al., 1991). Recently, allergens cross-reactive with Lol p 1 and Lol p 5 have been detected in pollen of other grass genera (Matthiesen and Løwenstein, 1991; Avjioglu, 1992; van Ree et al., 1992; Avjioglu et al., 1993). While Lol p 1 homologues are found in all grass pollens tested, Lol p 5 homologues are apparently confined to the Pooideae group, which includes Kentucky bluegrass, orchard grass, timothy and Yorkshire fog.

The major allergen source in the outdoor environment is grass pollen. Many atmospheric particles, including grass pollen grains which are approximately 35 µm in diameter, impact on the eyes and upper respiratory tract. They are too large to penetrate to the lower airways. Only recently, the mechanism by which grass pollen allergens penetrate to the lower airways to cause attack of asthma has been elucidated. This mechanism involves the interaction of IgE with allergen-containing micronic particles which are released from pollen grains on contact with moisture (Suphioglu et al., 1992). These submicronic particles and the mechanism for causing allergic symptoms are discussed in more detail later.

Weed aeroallergens: Of all the wind-pollinated weeds, that of most clinical importance is from short ragweed, *Ambrosia artemisiifolia* (family Asteraceae), which is the major cause of late summer hayfever in eastern North America (King et al., 1964, 1967; King, 1976), where ragweed accounts for up to 41% of the annual pollen catch (Dallas, Texas: Lewis et al., 1991).

In short ragweed pollen extract, 22 out of 52 proteins present have been identified as allergens by their reactivity with human IgE (Løwenstein and Marsh, 1983). The major allergen of short ragweed Amb a 1 (formerly antigen E) represents approximately 6% of the protein content of ragweed pollen (King et al., 1964), and 95% of ragweed-sensitive individuals show reaction to it in skin tests and have high IgE antibody titres with it (Zeiss et al., 1973). Other purified allergens of short ragweed pollen are Amb a 2, which is immunochemically related to Amb a 1 (King 1976; King et al., 1981), sharing the same mol. wt and antigenic determinants (King et al., 1967; Løwenstein et al., 1981), Amb a 3, Amb a 4, Amb a 5 and Amb a 6. In the soluble extract of ragweed pollen, Amb a 1 comprises 2.6%, of the soluble protein and Amb a 2 comprises 1.2%. Amb a 2 was identified as an important allergen based on its activity in skin tests and histamine release assays, which is about one-half that of Amb a 1, (King et al., 1967). Fifty to ninety percent of ragweed pollen allergic patients contain IgE that bind to Amb a 2.

Studies using skin tests with Amb a 1-depleted pollen extract led to the estimation that at least 90% of the total allergenic activity in ragweed pollen can be attributed to Amb a 1 (King et al., 1964; King, 1976). Competitive experiments using monoclonal antibodies to inhibit the binding of Amb a 1 by human IgE in ELISA assays have confirmed that Amb a 1 binds a substantial proportion of human ragweed allergic IgE (Olson and Klapper, 1986).

Homologues of Amb a 1 have been found in most members of the genus *Ambrosia* and in certain non-ambrosioid species. Studies of cross-reactivity among different ragweeds (short, giant, western and false) led to the interpretation that these ragweeds contain the same allergens in roughly comparable portions, whereas southern and slender ragweed possessed fewer of these allergens in lesser amounts. Other weed pollens which are considered to be clinically important include mugwort *Artemisia vulgaris* (Nilsen et al., 1991), *Parthenium hysterophorus* (Wedner et al., 1989), *Parietaria* (Corbi et al., 1986; Oreste et al., 1991) and *Plantago* (Watson and Constable, 1991).

Pollen grains from mugwort (*Artemisia*) are one of the main allergen sources in northern Europe (Charpin et al., 1974; Spieksma and Von Wahl, 1991), despite an incidence of only 1–4% of the annual pollen catch (Montpellier: Bousquet et al., 1984; Naples: D'Amato and Lobefalo, 1989). In Leiden, the Netherlands and Montpellier, France, 4 and 9%, respectively, of the pollen-sensitive patients have shown positive skin tests to *Artemisia pollen* (Bousquet et al., 1984; Spieksma, 1986). In mugwort pollen extract, ten of the 40 different proteins are allergenic (Ipsen et al., 1993).

In the Mediterranean region, pollen allergens from different species of *Parietaria* are very common, reflecting the high incidence of pollen in the atmosphere. In Naples, Italy, *Parietaria* (family Urticaceae) pollen accounts for 42% of the annual pollen catch (D'Amato and Lobefalo, 1989), while in Montpellier, France, there is only 4% (Bousquet et al., 1984). In Italy, 40–50% of pollen allergic adults are sensitive to *Parietaria* (Accorsi et al., 1980; Geraci et al., 1981). In *Parietaria judaica* pollen extract, nine of 28 proteins have been identified as allergens by their reactivity with human IgE (Ford et al., 1986).

2.2. Aeroallergens from seeds

After successful fertilization, seeds are formed, which have to be harvested, processed and transported to their destinations as a source of food. The resultant products are flour and bran (husk). During milling, transport and storage, the dust enters the atmosphere, causing both occupational and environmental asthma.

A well-documented case of occupational asthma to soybean flour, which contains nine allergenic proteins ranging from 14 to 55 kDa is provided by the work of Bush et al. (1988). A worker in a food-processing plant

where soybean flour was used as a protein extender, developed asthma after 6 years and suffered from symptoms of sneezing, coughing and wheezing within 15 min of exposure to soybean flour (Bush et al., 1988). The worker's serum specific IgE to soybean flour proteins, as assayed by direct RAST, was six times greater than that of a normal control serum. In addition, the sera contained specific IgE toward nine out of the 24 proteins of soybean flour extract.

Environmental asthma is also caused by soybean dust during transportation. In Spain, during the unloading of soybeans in the harbour, soybean dust became airborne causing epidemics of asthma (Anto and Sunyer, 1986; Anto et al., 1989; Hernando et al., 1989; Gonzalez et al., 1992; Anto et al., 1993; Navarro et al., 1993). The soybeans were unloaded by a vacuum system, but the silos did not have dust filters installed in the collection system, so that large quantities of soybean dust were released into the air (Anto et al., 1989). In a study of patients during a major asthma epidemic in Barcelona, Rodrigo et al. (1990) observed that 90% of the sampled patients' sera contained specific IgE to soybean hull allergens. During this period, the allergen levels determined using a high volume sampler and IgE from sera of allergic patients were high (1600–10 600 units), compared to the low values (0.3–320 units) observed in the air on non-epidemic days (Aceves et al., 1991).

The levels of IgE were more than 200% that of the serum pool from normal control: five subjects had a level more than 400% and three had ten times the values of normal control subjects. One hundred percent of these patients' sera reacted with allergens in the dust (IgE more than 200% of control). In addition, most of these patients' sera reacted strongly to allergens from hull and dust than other soybean extracts such as uncleaned and cleaned soybeans, and processed soybean meal and flakes. The major allergens in the hull and dust are glycoproteins of more than 14.4 kDa (Rodrigo et al., 1990; Swanson et al., 1991). The low mol. wt allergens are found in small amounts in all parts of soybean plants and at every stage of development, and are particularly high in pod and hull before the bean is mature, reaching the highest concentration in the hull at harvest time (Swanson et al., 1991). Indeed, the extract of soybean hull collected during the Barcelona asthma endemic was the richest source of allergens reacting with IgE from patients' sera.

Cereal grains are a common source of airborne allergens during storage, transport and the flour-milling process, and the source of 'baker's asthma' (Baldo et al., 1982). While several types of wheat flour proteins have been implicated as allergens, the major IgE-binding proteins appear to be members of a 12–15-kDa family of amylase or trypsin inhibitors (Walsh, 1988; Gomez et al., 1990).

The seeds of *Psyllium*, a member of the Plantago family, contained natural fiber that is used in laxatives

and cereal. *Psyllium* allergens can be dispersed in the air as dust during the mixing and transfer of the powder in factories. In an epidemiological survey of a pharmaceutical company, Bardy et al. (1987) showed that 32% (32 out of 120) of the workers have at least either a positive skin test or increased specific IgE to *Psyllium*. Extracts of three *Psyllium* seed components (husk, endosperm and embryo) have been shown to contain proteins that reacted with IgE in the sera of *Psyllium*-sensitive patients, as judged by crossed radioimmuno-electrophoresis and immunoblotting (Arlain et al., 1992).

2.3. Latex allergens

Natural rubber (*Hevea brasiliensis*) products such as surgical latex gloves, household gloves, and balloons can cause an allergic reaction in the predisposed human. The latex allergens are elutable from gloves under relatively mild conditions (Slater, 1992) and transferred to glove powder which becomes airborne (Lagier et al., 1990). Turjanmaa et al. (1990) used immunoblotting and IgE from a latex-allergic individual to identify the presence of similar allergens in ammoniated natural latex and eluates from surgical gloves and glove powders. Immunoblotting of natural rubber sap and individual patient sera showed that two major allergens, 14 and 21 kDa, existed. All the 14 patients' sera possessed IgE that bound to the 14-kDa allergens, whereas only six (40%) recognized the 21-kDa allergens (Alenius et al., 1991; Slater, 1991).

2.4. Enzymic aeroallergens of plant origin

Proteolytic enzymes are utilized for therapeutic purposes, in treatment of food (meat, beer), in research, and in the detergent industry. Proteolytic enzymes of plant origin, such as bromelain (pineapples) and papain (papaya), present in the form of dust, have been shown to cause allergic diseases after inhalation (Galleguillos and Rodriguez, 1978; Baur, 1979; Baur and Fruhmman, 1979a; Keenan et al., 1979; Gailhofer et al., 1988). Bromelain is extracted from fruit and leaves of plants while papain is a sulphydryl protease extracted from the latex of papaya fruit (*Carica papaya*). The allergenic activity is not dependent on their proteolytic capacity. Flindt (1979) has shown that a heat-inactivated solution of papain can still evoke positive skin prick test reactions in sensitized individuals.

In a survey of workers in an enzyme-handling factory, Flindt (1979) observed that of eight workers tested, seven gave positive skin prick test reactions to papain. In another population study of workers in meat tenderizing and pharmaceutical industries, Baur and Fruhmman (1979b) showed that seven out of 11 workers developed immediate hypersensitive reactions, such as hayfever and asthma. These workers have positive skin and RAST tests to papain compared to asymptomatic workers and non-exposed asthmatics. Other studies showed that 17 out of 33 workers exposed to airborne

papain at work experienced asthmatic symptoms, and the incidence of IgE-mediated sensitivity was 34.5% among the same group of workers (Baur et al., 1982). In a meat industry, where the enzymes papain, bromelain and ficin were used, 23% of 91 workers participating in a cross-sectional study had a positive skin reaction to one or more enzymes (Galson et al., 1988).

A study of occupational allergy in a beauty salon worker showed that papain powder can induce an allergic reaction (Niinimäki et al., 1993). The patient prepared an abrasive cream by mixing two powders (one of which was papain) into a moisturizing cream in a small room without ventilation. Allergic reactions were experienced after exposure for a year.

2.5. Allergens, particle size and site of deposition in the respiratory tree

Most pollen and spore aeroallergens are contained within particles of diameter greater than 10 μm . This physical characteristic strongly influences the site of deposition in the respiratory tree. Most pollen grains are too large to be respired (Hatch and Gross, 1964; Hoehne and Reed, 1971; Wilson et al., 1973); for example, grass pollen grains are $\sim 35 \mu\text{m}$ in diameter and essentially are all deposited in the nose and oral cavity. However, a number of hay fever patients develop asthma during the grass pollen season. The question of how allergens from pollen grains can become respirable and trigger asthma will now be considered.

Pollen allergens are known to occur naturally in the atmosphere associated with much smaller particles than the grains (Solomon et al., 1983; Stewart and Holt, 1985; Schumacher et al., 1988; Spiekma et al., 1990). These allergen-containing particles range in size to within the respirable range, that is, $< 5 \mu\text{m}$. Such inhalable allergen-containing particles are known as micronic particles (Busse et al., 1972). For grass pollen, micronic particles have been detected in the atmosphere of European and Australian cities during the grass pollen season (Stewart and Holt 1985; Paggiaro et al., 1990; Spiekma et al., 1990; Suphioglu et al., 1992). Similarly, micronic particles containing ragweed allergens (Busse et al. 1972; Habenicht et al. 1984), oak (Fernandez-Caldas et al., 1989) and birch allergens (Rantio-Lehtimäki, 1994) exist in aerosol fractions smaller than intact pollen grains. Micronic particles have been detected using different kinds of air samplers and evaluation of particle size distribution (Agarwal et al., 1981; Solomon et al., 1983; Stewart and Holt, 1985; Jensen et al., 1989; Spiekma et al., 1990). A feature of the grass pollen season is that concentrations of micronic particles increase significantly on days following rainfall (Stewart and Holt, 1985; Suphioglu et al., 1992).

The origins of these micronic particles were unknown until recently (Spiekma et al., 1990). Several possibilities are now considered.

Pollen fragments: These may be produced by physical degradation during deposition and redeposition in the atmosphere (Knox, 1979; Solomon 1984, 1986).

Orbicules (Utricle bodies): These small spheres, $< 0.1 \mu\text{m}$ in diameter, coat the tapetal membrane (inner anther lining) in several allergenic plants, for example, grasses (Knox 1984b), ragweed and Japanese cedar. They are made of sporopollenin, like the exine and may fragment from the anther lining and be released in the atmosphere (see Knox, 1984b). The major allergen of Japanese cedar pollen, Cry j 1 has been shown by immunogold labelling following rapid freeze-substitution, to be present in the orbicules (Miki-Hirosige et al., 1994).

Microdroplets: Atmospheric aerosols may contain allergen molecules released from airborne pollen grains in nature (Solomon, 1984, 1986)

Allergen molecules bound to fine particles less than 2.5 μm : Allergen molecules in aerosols may bind to fine physical particles, for example, soot (carbon), of various sizes in the atmosphere. This is of particular relevance for atmospheric pollution in urban areas, where diesel vehicle emissions include fine soot particles in a size range less than 2.5 μm . Such inhalable particles in the atmosphere of US cities have been associated with increased morbidity (Dockery et al., 1993), presumably due to the presence of carcinogens, such as benzene, bound to the particles. They also provide a site for binding of allergen molecules in the atmospheric aerosol, providing a source of micronic particles in polluted areas.

Allergen-containing starch granules are a source of micronic particles from grass pollen: Starch granules from grass pollen of a diameter within the respirable range, 0.5–2.5 μm , represent a recently discovered source of inhalable allergens (Singh et al., 1991; Knox, 1993). About 700 starch granules can be released from each rye-grass pollen grain following osmotic rupture during rainfall. This source was discovered during molecular biological investigations of the genes encoding grass pollen allergens. The gene encoding Lol p 5 contains a signal peptide with amino acid motifs similar to those of chloroplast transit peptides, suggesting that the allergen is synthesized in the cytosol of developing pollen and targeted into amyloplasts, a type of chloroplast (Singh et al., 1991). Immunogold localization with Lol p 5-specific monoclonal antibodies located the allergen within intracellular starch granules, which are formed in amyloplasts. Isolated starch granules have been shown to be allergenic and contain Lol p 5 (Suphioglu et al., 1992). Homologous allergenic protein in timothy grass, Phl p 5, has also been shown to be present in starch granules (Grote et al., 1994). Starch granules have been detected in the atmosphere during the grass pollen season on both dry sunny days and on days following rainfall when there was a 50-fold increase in starch granule numbers m^{-3} of air. Heavy rainstorms are associ-

ated with epidemics of thunderstorm asthma in Melbourne during the grass pollen season, and all members of a sample of the asthmatics responding proved to be sensitised to grass pollen (Bellomo et al., 1992).

When grass pollen grains rupture, they release not only starch granules but all their cytoplasmic organelles, nuclear and cytosolic contents. These include numerous P-(polysaccharide) particles, which are derived from Golgi vesicles, diameter 0.1–0.2 μm , composed of pectic polymers (see Knox, 1984a) and destined to form the pollen tube wall when the grains germinate. Allergen molecules in the aerosol could presumably bind to these P-particles, although there is as yet no evidence for this.

The presence of micronic particles in the air suggests that the true level of allergens in the atmosphere, that is, the allergen load, is represented by the sum of intact grass pollen grains, together with micronic particles. Thus, pollen counts do not necessarily give a precise evaluation of allergen load. Conversely, micronic particles cannot be routinely counted with a Burkard volumetric spore trap (Hirst, 1952; Ogden et al., 1974). Special quantitative methods have been developed to estimate allergen load and these will now be reviewed.

2.6. Allergen load analysis

Immunochemical analysis of allergen load in the atmosphere has given a more accurate picture of atmospheric allergens. Methods involve use of high volume air samplers to collect allergens in the air onto fiberglass filters, and the allergens are then eluted and assayed using RAST-inhibition. Three types of methods to elute allergens from fiberglass filters have been described (Agarwal et al., 1981; Jensen et al., 1989): homogenization, descending elution and recycling methods. Agarwal et al. (1981) reported that with ragweed pollen, descending elution gave a higher yield compared with the homogenization method. However, Jensen et al. (1989) found that with timothy grass pollen, homogenization gave the optimum yield along with greater convenience. In addition, they observed that in the descending elution method carried out for 8 h or longer, different allergens have different elution kinetics and degradation of allergens occurred. These results suggest that different pollen allergens require different methods of elution. This method also generates a high volume of liquid, so that lyophilization is necessary to concentrate the allergens, and with timothy grass allergens, loss of allergenicity occurred after lyophilization (Jensen et al., 1989). These methods of eluting allergens from fiberglass filters for RAST-inhibition assay are laborious and cumbersome. In addition, not all allergens on fiberglass filters are eluted and some of the allergens are enzymatically degraded or loss of allergenicity occurs during these manipulations.

Recently, Spieksma et al. (1990) reported an in-situ method of estimating airborne allergen load, without the need for eluting allergens. Allergen load is estimated by applying pooled human sera, containing specific IgE antibodies, onto a 20-cm long particle-loaded impaction strip. Serum is allowed to descend through the strip where specific IgE reacts with allergens and unbound specific IgE is collected, and measured using RAST-inhibition. Problems with this method include insufficient time (1 h) for the IgE-allergen interaction, so that allergens on the fiberglass filter may not be able to contact or bind IgE. Further, radioactive labelling of secondary antibodies are needed in the RAST-inhibition assay. In addition, use of a radioactive label is hazardous, expensive and has the problem of a short shelf-life. These methods may not give as precise an estimation of total airborne allergen load.

We have developed an improved IgE solid phase inhibition immunoassay, without the need for elution or radioactivity, for estimating grass pollen environmental allergen load using filters from high volume air samplers (Ong, 1994). This method involves the use of small circular disc samples cut from the filter, whose size is pre-determined to ensure a quantitative relationship with the IgE in the pooled sera. Fiberglass filters containing grass pollen allergens are used directly without any manipulation, resulting in accurate estimates of grass pollen allergen load. Our method makes use of enzyme labels and fluorescent substrates to enhance its sensitivity. This method is limited only by the availability of allergic sera containing IgE specific to the allergen and commercially available solid phase discs.

3. Conclusions

We have presented an update on the sources and molecular basis of aeroallergens of plants. These aeroallergens are a natural component of the atmosphere either because of their natural function or as a result of human activity. Pollen grains provide sources of allergens generally within the larger 10–200 μm size range, with only a few pollen types less than 10 μm . While most allergenic pollen types account for only 20–30% of the total annual pollen catch, during their flowering season they are usually the dominant type. We have shown that they possess a number of characteristic features.

- Tree pollen types commence the season in winter. For example, birch pollen counts in Scandinavia are the highest daily pollen counts yet reported (> 3000 grains $\text{m}^{-3} \text{day}^{-1}$). The major allergen, Bet v 1, is a 14-kDa protein that is similar in terms of its amino acid sequence to pathogenesis-related proteins, defence molecules of other plants (Breiteneder et al., 1989).

- Grass pollen follows in spring, and is unique as its two immunodominant allergens, Lol p 1, a 35 kDa glycoprotein, and Lol p 5, a 28–32-kDa protein, are in different cellular sites (Singh et al., 1991). Lol p 1 is synthesised in the cytosol, on the surface of pollen grains, and in intracellular starch granules. The allergens at the pollen surface are not inhalable and can interact only with the eyes, nasal and oral cavities. Starch granules, containing Lol p 5, are released to the atmospheric aerosol when grains rupture in rainwater. These are a major source of allergen-containing micronic particles, which are important because they are inhalable, and are prevalent following thunderstorms during the pollen season when they appear to be triggers of 'thunderstorm asthma' (Bellomo et al., 1992; Suphioglu et al., 1992). At the same time, allergen molecules are present in the aerosol, and these can bind to soot particles, and so be respired deep into the airways.
- The major Japanese cedar pollen allergen Cry j 1, has been detected both within the pollen and in orbicules, particles less than 1 µm that line the anther cavity and can be released into the air when dehiscence occurs (Miki-Hirosige et al., 1994).
- Ragweed is the major cause of late summer hayfever in eastern North America, where its pollen accounts for up to 41% of the annual pollen catch. It is a major source of aeroallergens, Amb a 1 and Amb a 5, in both respirable and non-respirable size ranges (Lewis et al., 1991).
- As a result of human activity, dusts derived from seeds and cereal grains during transport, storage, and milling provide a source of micronic particles containing potent allergens that can trigger allergic disease.

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