

Localization, release and bioavailability of pollen allergens: the influence of environmental factors

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Allergens are integral constituents of plants or animals and their normal functions and localization are being characterized. To trigger responses in humans, allergens must become bioavailable and the role of air pollutants – for example diesel-exhaust particles – in this process is causing concern. Finally, the fact that some pollen releases eicosanoid-like proinflammatory mediators may have wide implications.

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Introduction

Allergic diseases represent a major health problem of most modern societies [1–3]. According to the European Allergy White Paper [1], the overall prevalence of seasonal allergic rhinitis in Europe is approximately 15%. Current asthma prevalence rates vary from 2.5%–10.0% and the prevalence of allergic atopic dermatitis ranges from 9%–24%. Atopic diseases are still increasing, especially in regions with low prevalence rates to date — for example in the former East Germany [4]. The reason, however, remains still to be elucidated although a variety of explanations are under scientific discussion.

Allergies are among those few diseases in which environmental factors of both natural and anthropogenic origin have been identified as a cause of disease development, as well as of elicitation and aggravation of symptoms. Naturally occurring allergens from indoor and outdoor environments — that is, house-dust-mite, animal dander and pollen allergens — are in the very first line of substances inducing sensitization among young children from atopic parents [5]. Urban air pollution, especially pollutants due to traffic emissions, exhibits adjuvant activities in allergen-specific IgE production [6] and has been shown to increase prevalence rates of both sensitization [7,8*] and symptoms of allergic rhinitis in atopic children [8*].

It is commonly believed that allergic sensitization starts when an allergen gets into contact with antigen-presenting cells at sites of mucosal or skin epithelium. For elicitation of allergic symptoms and maintenance of the disease, the repeated contact of an allergen with the individual is the absolute prerequisite. This contact occurs via the air

through inhalation, via the gastrointestinal tract through ingestion and also through direct contact on the skin surface or percutaneously via injections. Most IgE-inducing allergens are environmental substances of animal or plant origin and are proteins or glycoproteins with a molecular weight >10 kDa [9,10]. These allergens are taken up by dendritic cells, processed and presented on their surface to T lymphocytes, which then — via the development of a Th2-cytokine secretion pattern — induce B cells to secrete IgE antibodies. This process — during which no symptoms of allergic disease are manifest — is called ‘sensitization’. Repeated allergen exposure of sensitized individuals leads then to the elicitation of allergic symptoms of disease such as wheal-and-flare reactions or eczema in the skin, rhinoconjunctivitis, sneezing or airway bronchoconstriction.

Some allergens are more potent in inducing IgE formation than others; the difference in this allergenic potency is, however, not well understood at a molecular level. Some allergens have enzyme activity, others do not [9–11,12*]. Allergens from birch or grass pollen have a rather high allergenic potency, whereas allergies against pine pollen are extremely rare in Central Europe, although there is a tremendous amount of pine pollen in the outdoor air during spring and early summer.

Exposure to inhalant allergens occurs through the air. Allergens are, however, not freely available but are an integral constituent of plants or animals — the allergen carriers — and have to be bioavailable in order to provoke an allergy. In the outdoor and indoor environment, allergen carriers are mainly represented by pollen, mold spores, dust mites and animal epithelia. The most important allergen carriers in the outdoor air is pollen — with a diameter between 15 μm and 60 μm — from anemophilic plants such as trees, grasses and weeds. Pollen counts and pollen-count forecasts are available in most countries and document the amount and nature of different pollen species over the year in the respective areas [13].

During the past ten years knowledge about the molecular biology of allergens and its implications on the pathomechanism of allergic inflammation, as well as on the development of new therapeutic strategies, has been greatly advanced. This review covers the increasingly interdisciplinary information available to date on allergen localization and release from its carriers and on factors influencing allergen bioavailability in the development of IgE-mediated allergic diseases.

Allergens as integral constituents of pollen

The most frequent aeroallergens derive from pollen. Among them, grass pollen represents the biggest allergen source.

Table 1

Molecular characterization of the most relevant grass pollen allergens.

Allergen name	Species of grass	Type of grass	Protein function (if known)	Molecular weight (kDa)	Sequence/structure*	Is the recombinant protein available?	Accession number†
Dac g 1	<i>Dactylis glomerata</i>	Orchard grass		32	p	No	–
Dac g 2	<i>Dactylis glomerata</i>	Orchard grass		11	c	Yes	S45354
Dac g 3	<i>Dactylis glomerata</i>	Orchard grass		14	c	Yes	A60359
Dac g 5	<i>Dactylis glomerata</i>	Orchard grass		31	p	No	–
Hol l 1	<i>Holcus lanatus</i>	Velvet grass		34	c	Yes	Z27084
Hol l 5	<i>Holcus lanatus</i>	Velvet grass	RNase I	30	c	Yes	Z97874
Lol p 1	<i>Lolium perenne</i>	Rye grass		27	c	Yes	M57476, M57474
Lop p 2	<i>Lolium perenne</i>	Rye grass		11	c	Yes	A34291
Lop p 3	<i>Lolium perenne</i>	Rye grass		11	c	Yes	A33422
Lop p 4	<i>Lolium perenne</i>	Rye grass		?		Yes	A60737
Lop p 5a	<i>Lolium perenne</i>	Rye grass		?	?	No	–
Lop p 5b	<i>Lolium perenne</i>	Rye grass		31,35	c	Yes	S38289, S38290
Lop p 10	<i>Lolium perenne</i>	Rye grass	Cytochrome C	?	?	No	–
Lop p 11	<i>Lolium perenne</i>	Rye grass	Glycoprotein	18	c	No	–
Phl p 1	<i>Phleum pratense</i>	Timothy grass	Protease	35	c	Yes	X78813
Phl p 2	<i>Phleum pratense</i>	Timothy grass		11	c	Yes	X75925
Phl p 3	<i>Phleum pratense</i>	Timothy grass		11	c	No	–
Phl p 4	<i>Phleum pratense</i>	Timothy grass		50	p	No	–
Phl p 5a	<i>Phleum pratense</i>	Timothy grass		38	c	No	X70942
Phl p 5b	<i>Phleum pratense</i>	Timothy grass	RNase	32	c	Yes	Z27083
Phl p 6	<i>Phleum pratense</i>	Timothy grass		14	c	Yes	Z27082
Phl p 7	<i>Phleum pratense</i>	Timothy grass	Ca ²⁺ -binding allergen	8,6	c	Yes	O82040
Phl p 12	<i>Phleum pratense</i>	Timothy grass	Profilin	14	c	Yes	P35079
Phl p13	<i>Phleum pratense</i>	Timothy grass	Polygalacturonase	55–60	c	Yes	AJ238848
Poa p 1	<i>Poa pratensis</i>	Kentucky blue grass		33	c	Yes	A60372

*For most grass pollen allergens, complete (c) or partial (p) protein sequences/structures are known. †Accession numbers are for sequence databases such as that of the European Bioinformatics Institute (EBI; <http://www.ebi.ac.uk>).

Since grass pollen is able to evoke IgE-mediated allergic reactions within seconds after contact with the mucosa, pollen allergens must be extremely water soluble and readily available. Allergens are non-self proteins with antigenic properties and molecular weights of >10 kDa. They are not only able to elicit an IgE response but also trigger mediator release from mast cells or basophilic cells through bridging two cytotoxic IgE molecules via FcεRI receptors. This means that an allergen must possess at least two IgE-reactive epitopes. In practice, allergens are identified on the basis of IgE reactivity, which is sufficient for most purposes [14].

Allergen nomenclature follows the rules of the IUIS (International Union of Immunological Societies) Allergen Nomenclature Subcommittee ([15]; <http://www.allergen.org/List.htm>). Designations are made on the basis of the taxonomic names of the genus and species from which the allergens are derived: the designations comprise the first three letters of the genus, a space, then the first letter of the species, another space, then an Arabic numeral according to priority of discovery and description, unless the allergen is structurally homologous and immunologically cross-reactive with previously described allergens from different species, in which case an existing numeral is assigned; for example, in grass pollen, predominantly grass group 1 and group 5 allergens exist.

The individual allergens in a representative diagnostic extract of the allergenic source are often classified as being of major (>50% frequency), intermediate or minor (<10% frequency) importance on the basis of the frequency with which they are recognized by patients allergic to the respective allergenic source material [16].

The specificity of the allergic reaction is strongly correlated with the chemical structure of the allergens. The easiest way to elucidate the primary protein structure of allergens is via recombinant proteins derived using the DNA sequences of cloned allergens. Table 1 depicts the actual status of identified and structurally characterized grass pollen allergens.

The development of specificities to allergens depends primarily on the individually conditioned immune system of each patient. The immune system is not able to recognize the whole structure of macromolecules such as proteins or glycoproteins but recognizes smaller sections — called determinants or epitopes — of the molecules. It cannot be excluded, however, that there is an interdependence of the three-dimensional presentation of the epitope with the whole structure of the molecule. A given epitope may therefore display a different immunological behaviour depending upon the whole structure of the molecule. In principle, the immune system reacts with two different

kinds of epitopes: conformational epitopes that are made up of the (three-dimensional) surface area of a molecule formed by discontinuous sections of the primary protein structure; or linear ones that are formed directly by the primary protein structure.

Three compartments of the immune system deal with epitopes: B cells and their secreted antibodies; T cells via the TCR; and cells presenting epitopes on molecules of the MHC. Whereas B cells react to both conformational and linear epitopes, T cell epitopes are exclusively of a linear nature. MHC class I molecules react with 9-mer peptides via anchor points in position 2 and 9; MHC class II molecules react with peptides, which are more heterogeneous in length, via anchor points in the relative positions 1, 4, 6 and sometimes 9 [17].

Normally allergens, especially aeroallergens, possess conformational epitopes that are rather susceptible to denaturing influences in that they lose their IgE-reactivity.

The localization and visualization of grass pollen allergens

Pollen grains are considered as the multicellular male gametophyte in both angiosperms and gymnosperms. Upon contact with the receptive surface, the stigma, pollen germinates by tube development [18]. Of allergological relevance are the anemophilous pollen from gymnosperms that is wind-dispersed and transported over wide areas. Grass pollen, for example, can be transported over about 175 km at an average wind-speed of 10 m/second. They sediment in motionless air about 3.1 cm/second [18]. One-tenth of the >250 000 pollen-producing species known in the world are wind-dispersed and of allergological relevance [13].

Pollen grains are sealed in a double-layered wall. Its outer layer, the exine, not only displays unique, species-specific morphological features but also is composed out of a lipophilic sporopollenin, which is extremely resistant to exogenous influences. This unique property of the pollen wall is the basis for the fact that pollen can survive for centuries. The softer inner layer, the intine, encloses the pollen cytoplasm containing all subcellular organelles, including a vegetative and a generative nucleus as well as starch granules and polysaccharide (p)-particles (Figure 1). Pollen exine is interrupted by pores or apertures through which tube formation is initiated. In addition, small canaliculi connect the outer pollen surface with its intine.

Since pollen allergens are highly water-soluble proteins that can be released into a humid milieu within seconds, their localization *in situ* requires special fixation and dehydration procedures [19•] prior to visualization by fluorescence-labeled or immunogold-labeled antibodies in light and/or electron microscopy. Knox and coworkers were the first to identify sites that bind anti-allergen monoclonal antibodies recognizing group 1 and group 5 allergens in

pollen of the rye grass, *Lolium perenne* [20–25]. Later, Grote *et al.* demonstrated, in a series of papers (for references, see [19•]), antibody-binding sites for various major allergens from birch and grass pollen (i.e. group 1 allergens, group 5 allergens, Phl p 4, Phl p 6) after anhydrous fixation techniques and electron microscopy. Behrendt *et al.* [26,27] were able to identify antibody-binding sites in semithin sections of *Phleum pratense* pollen for the first time by the use of strictly anhydrous preparation protocols and the immunogold-labeling technique as well as by the use of cryosectioned samples visualized by fluorescence-labeled monoclonal antibodies against two major allergens, Phl p 1 and Phl p 5 [27].

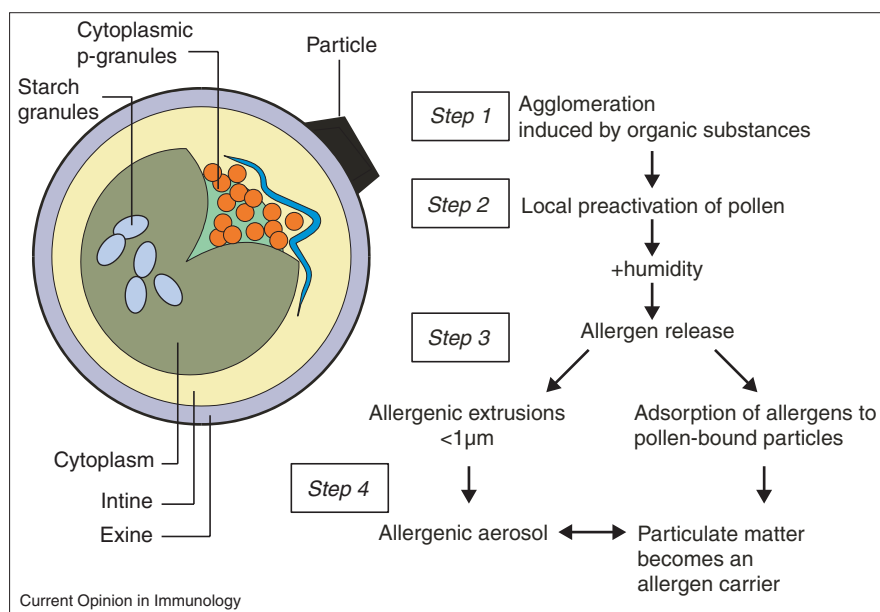
It was found that in dry, unhumidified pollen, all allergens investigated so far showed little activity within the cytoplasmic matrix, but strong activity within organelles such as mitochondria, p-particles and starch granules. Group 5 allergens are predominantly mapped to the amyloblasts (starch granules) but not to the pollen wall [25,28]. A group 1 allergen in *L. perenne* pollen was detected in the cytoplasmic matrix and in mitochondria, in *P. pratense* pollen only in the matrix. Using slightly different but strictly anhydrous fixation protocols and different monoclonal antibodies, a group-5 allergen in *P. pratense* pollen, Phl p 5, was shown to be present in the cytoplasmic matrix and in the exine, but not in starch granules [26,27]. Furthermore, Phl p 1 allergen has been shown by the same authors to mainly occur within the cytoplasm and in the intine [27] but not on the exine. These findings are consistent with the identification of Phl p 1 as a novel expandin-like molecule with cysteine-protease activity [29], which is physiologically activated during early events of tube formation. Other grass pollen allergens such as Phl p 4 and Phl p 6 were found to occur at matrix sites and in starch granules, and within the p-particles of the cytoplasm, respectively [30,31].

Allergen liberation from pollen grains and the bioavailability of aeroallergens

Allergen liberation from sites within the pollen is a prerequisite for its function in sensitized individuals. In a dry atmosphere, pollen is very stable and can contain their allergens over centuries [18]. There are at least three inducers of allergen liberation: first, a high relative air humidity; second, thunderstorms and heavy rain; and third, air pollutants. Under humid conditions, allergens are released from pollen grains in the process that occurs under the physiological conditions of pollination [26].

This liberation of allergens is pH- and temperature-dependent, with maximal values at 37°C and pH 9.0 for total protein release from birch, grass and pine pollen and pH 7.4 for liberation of the major allergens Bet v 1 and Phl p 5 [27]. About one-third of the total protein mass released from birch pollen is Bet v 1, as determined by ELISA. In contrast, only 10% of the total proteins released from grass pollen display the major allergen Phl p 5 [27]. In an early study [32] the mechanism of protein release from grass pollen *Dactylis*

Figure 1



Proposed mechanism of allergen-containing aerosol production through pollen-particle interaction. On the left is a schematic of a pollen grain with a particle attached to it; the various steps in the generation of allergenic aerosols are shown on the right.

glomerata after a two-hour incubation period of pollen with ammonium carbonate buffer — a method to obtain extracts of high allergen content — was investigated by conventional transmission and scanning electron microscopy. In this study, transmission electron microscope analysis revealed a reduction of electron density and lamellation of the intine, fusion of cytoplasmic p-particles and exposure of granular matrices to the intine (see also Figure 1). In addition, granular material appeared outside the exine, just on top of microchannels — indicating liberation of probably allergenic material. This was also shown ten years later — by means of immunogold field emission scanning and transmission electron microscopy — for hydrated rye-grass pollen [33[•]]. Additionally, expulsion of cytoplasmic material through the germination pore gives rise to numerous starch granules and small roundish bodies in the vicinity, indicating the morphological *in vitro* equivalent of thunderstorm situations with heavy rainfall [33[•]].

In rare situations — for example during a thunderstorm — pollen may break by osmotic shock, releasing allergen-containing starch particles. This finding might explain the higher frequency of asthma attacks during thunderstorms as reported in Australia (for references, see [34,35]). Results from a case/control study performed in six towns in South-Eastern Australia support the hypothesis that epidemics of asthma exacerbations are caused by high concentrations of allergenic particles [36[•]]. These particles are postulated to be produced “by an outflow of colder air, associated with the downdraught from a thunderstorm, sweeping up pollen grains and particles and then concentrating them in a shallow band of air at ground level” [36[•]]. These positive associations between the frequency of asthma attacks, thunderstorms and high allergen concentrations seem to be restricted to the

Australian continent. In England, acute asthma epidemics are not associated with thunderstorms or unusual weather conditions [37] although thunderstorms have been proposed to be a risk factor for asthma attacks [38].

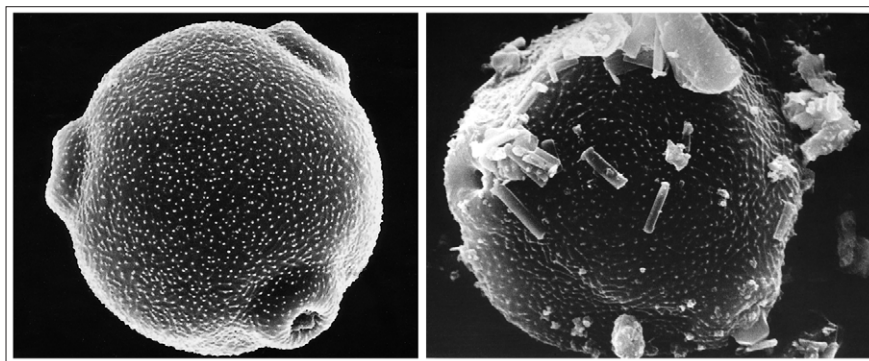
Allergen liberation from pollen grains can occur in two different compartments: first, on the mucosal surface of the upper respiratory tract after exposure to pollen; and second, outside the individual organism in the ambient air itself. Allergenic activity has indeed been detected by several groups in air samples and fractions below 1 µm in diameter [39–44]. The biological relevance of this second allergen-containing compartment in the outdoor air has been extensively reviewed by Monn and Koren [45[•]]. The existence of two different allergen-containing compartments in the ambient air — pollen grains themselves as well as allergenic aerosols — may explain why, unlike in the indoor environment with house dust mite allergens, the risk of sensitization against outdoor allergens is not yet adequately estimated by simple pollen counts. Methods for the quantitative evaluation of allergen content in the outdoor air are required [7,27,45[•]].

The influence of air pollutants on allergen bioavailability and on other allergy-relevant factors from pollen

By the use of fluidized bed reactors, the influence of both gaseous and particulate air pollutants upon allergen release from pollen can be studied in a dose-, time- and humidity-dependent fashion [26,46]. Exposure of *P. pratense* pollen to high concentrations of SO₂ induces a significant reduction of liberation of a major allergen, Phl p 5 [26], thus leading to the assumption that the bioavailability of major grass pollen allergens might be grossly reduced in regions

Figure 2

Scanning electron micrographs of birch pollen from a rural (left) and an urban (right) collection site. The latter is reproduced, with permission, from [7].



polluted with SO_2 as compared with non-polluted areas. This observation might help to explain the well-known paradoxical finding of low asthma and hay-fever rates in 5–6-year-old children from areas heavily polluted with SO_2 in the former East Germany in 1991 compared with children from West Germany [47,48]. When, under the same conditions, pollen grains were exposed to NO_2 — a major indoor and outdoor contaminant — no change in allergen bioavailability was seen [26].

Pollen that had been collected at road-sides that were heavily polluted with traffic-related emissions, however, also showed significantly reduced allergen release in comparison with pollen collected in rural meadows [27]. Since traffic-heavy areas are associated with high prevalence rates of allergic diseases of the upper airways, the findings might indicate the continuous release of allergen from pollen and the binding of free allergen to airborne particles — that is, diesel-exhaust particles. Binding of highly purified samples from the natural major grass pollen allergen, Lol p 1, to diesel-exhaust particles under experimental conditions has indeed been observed by Knox *et al.* [49•]. Furthermore, airborne particles agglomerate onto pollen surfaces in traffic-heavy areas but not in park regions that are devoid of high concentrations of air pollution [50]. Particle agglomeration onto pollen surfaces is a common feature of pollen that has been collected in polluted areas (Figure 2).

Today, particulate ambient-air pollutants, especially diesel-exhaust particles, are of major concern. Airborne particulate matter in the Western industrialized world is composed of more than 700 organic substances adsorbed onto particles whereas the particle core itself consists of either minerals such as silica, iron, aluminium, magnesium, sulfur, manganese and lead, or of carbonaceous organic material. Particle-adsorbed organic substances mainly belong to the group of polycyclic aromatic hydrocarbons, phenols or aza-heterocyclic compounds [7]. These substances have been shown to mediate pollen–particle interaction [32]. When grass pollen from *D. glomerata* was incubated with aqueous extracts of airborne particulate matter, morphological alterations with characteristics seen

during the activation of pollen under humid conditions are seen. At the same time these pollen grains release substantial amounts of proteins in a dose- and time-dependent manner [26]. Pollen treated with organic extracts of atmospheric fine dust, however, displays signs of local pollen pre-activation [32] but no release.

These findings led to the hypothesis that allergen-containing aerosols are generated through pollen–particle interaction in a moist atmosphere (Figure 1). This view is further supported by scanning electron microscopy: pollen exposed to extracts of airborne particles is characterized by the appearance of small polygonal particles on the pollen surface that are similar to allergen-containing particles extruded from hydrated pollen [33•]. These particles, much smaller than 1 μm , belong to the submicronic fraction and resemble those seen on the surface [32] of pollen collected in regions highly polluted with combustion products ([7,32,50]; Figure 2). The interaction of pollutants with pollen in the ambient air induces not only quantitative but also qualitative changes with regard to pollen morphology, protein and allergen release, and the generation of particulate allergen-containing aerosols [26,27,32,45•].

Pollen grains do not only release protein and allergen under humid conditions. Recently we reported the surprising finding that pollen grains are able to secrete significant amounts of eicosanoid-like substances (i.e. crossreacting with leukotriene-B4 and prostaglandin-E2 in enzyme immunoassays) in a pH-, time- and temperature-dependent fashion [51•]. The highest values were found in birch, grass and mugwort pollen, whereas pine pollen showed only marginal leukotriene-B4-like secretion. The release of these proinflammatory substances was significantly higher from pollen that had been collected near roads with heavy traffic, indicating a stronger proinflammatory activity of these pollen grains [51•].

These findings, which have to be further studied in detail, may have wide implications. They open a new dimension in the understanding of the early events in both allergic sensitization ('initiation of sensitization') and exacerbation

of the disease prior to allergen-induced mechanisms. The allergen carrier — the pollen grain — can contribute by itself to the activation of the mucosal epithelium of the respiratory tract by secreting proinflammatory mediators. It is especially remarkable that pollen grains from plants with high allergenicity (e.g. birch, grass and mugwort) produce significantly higher amounts of these proinflammatory substances than pine pollen, which exhibits much less pronounced allergenicity. Thus, the term 'allergenic potency' should not only be considered at the molecular level of allergen structure but also include the characteristics of the allergen carrier and its ability to enhance sensitization by directly activating inflammatory cells of the animal. This process of initiation of sensitization precedes the contact between allergen and antigen-presenting cell and may be the very first step in atopic sensitization, which later comprises T-cell activation, antibody production, development of immunological memory and recruitment of cells that amplify the immune response.

Conclusions

Pollen allergens are integral pollen constituents. They are primarily localized within the pollen grain in specific patterns of distribution and have to be released during a process of activation in order to become bioavailable. Allergen liberation does not depend on cell death but is a temperature-, time- and pH-dependent active mechanism.

Environmental factors — such as humidity, thunderstorms and air pollutants — have been shown to influence allergen liberation in a complex manner. Among gaseous pollutants, SO₂ has a clear-cut inhibitory effect on allergen release, a phenomenon that may contribute to the well-known fact of decreased allergy prevalence in regions highly polluted with SO₂. Other gaseous pollutants (e.g. NO₂) have almost no effect.

In highly polluted regions, windborne pollen grains not only carry allergens, but also interact with pollutants. Both organic and aqueous extracts from particulate air pollutants have been shown to activate pollen and induce protein and allergen release as well as morphological changes on the pollen surface. This interaction between particles and pollen in the atmosphere may lead to pollen pre-activation and agglomeration, and to modulation of allergen release, generation of allergenic aerosols and adsorption of pollen proteins to airborne particles.

The finding that, under humid conditions, pollen grains themselves secrete proinflammatory eicosanoid-like substances that are able to attract and activate inflammatory cells opens a new dimension to our understanding of the early events in allergic sensitization. It may help to redefine the term 'allergenic potency' of various allergens.

The aspect of allergen bioavailability should be considered more thoroughly in the future since it seems to be an important factor in establishing a dose/response relationship for outdoor allergen exposure and disease outcome.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Aas K, Åberg N, Bachert C, Bergmann K, Bergmann R, Bonini S, Bousquet J, de Weck A, Farkas I, Hejdenberg K *et al.*: *European Allergy White Paper: Allergic Diseases as a Public Health Problem*. Brussels: The UCB Institute of Allergy, Brussels; 1997.
2. Behrendt H, Ewers HJ, Hüttl RF, Jänicke M, Plaßmann E, Reh binder E, Sukopp H: (*Der Rat von Sachverständigen für Umweltfragen*). *Umwelt und Gesundheit. Risiken Richtig Einschätzen. Sondergutachten*. Stuttgart: Metzler-Poeschel; 1999. [Title translation: (*The Council of Experts for Questions Regarding the Environment*). *Environment and Health. Calculate Risks Correctly. Special Report.*]
3. Ring J: **Allergy and modern society: does 'Western life style' promote the development of allergies?** *Int Arch Allergy Immunol* 1997, **113**:7-10.
4. Mutius E, von Weiland SK, Fritzsche C, Duhme H, Keil U: **Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany.** *Lancet* 1998, **351**:862-866.
5. Anonymous: **Allergic factors associated with the development of asthma and the influence of Cetirizine in a double-blind, randomized, placebo-controlled trial: first results of ETAC (Early Treatment of the Atopic Child).** *Pediatr Allergy Immunol* 1998, **9**:116-124.
6. Diaz-Sanchez D, Tsien A, Fleming J, Saxon A: **Combined diesel exhaust particulates and ragweed allergen challenge markedly enhances human *in vivo* nasal ragweed-specific IgE and skews cytokine production to a helper cell 2-type pattern.** *J Immunol* 1997, **158**:2406-2413.
7. Behrendt H, Friedrichs KH, Krämer U, Hitzfeld B, Becker WM, Ring J: **The role of indoor and outdoor air pollution on allergic diseases.** In *Progress in Allergy and Clinical Immunology*, vol 3. Edited by Johansson JO. Seattle: Hogrefe & Huber; 1995:83-89.
8. Krämer U, Koch T, Ranft U, Ring J, Behrendt H: **Traffic-related air pollution is associated with atopy in children living in urban areas.** *Epidemiology* 2000, **11**:64-70.
- This is the first epidemiological publication of a positive association between health-outcome variables — symptoms of allergic rhinitis (reported using a daily questionnaire) as well as prevalence of pollen sensitizations — and measurements of traffic-related air pollution. The authors report about association of allergies of the upper airways with outdoor concentrations of NO₂ exposure. They conclude that monitoring outdoor NO₂ is a good indicator for assessment of traffic-related air pollution but not the cause of the observed effects.
9. Becker W-M: **Allergen epitopes.** In *New Trends in Allergy*, vol 4. Edited by Ring J, Behrendt H, Vieluf D. Berlin: Springer; 1997:339-344.
10. Bufe A, Spangfort MD, Kahlert H, Schlaak M, Becker WM: **Major birch pollen allergen Bet v 1 shows ribonuclease activity.** *Planta* 1996, **199**:413-415.
11. Schramm G, Petersen A, Bufe A, Schlaak M, Becker WM: **Identification and characterization of the major allergens of velvet grass (*Holcus lanatus*), Hol I 1 and Hol I 5.** *Int Arch Allergy Immunol* 1996, **110**:354-363.
12. Suck R, Petersen A, Hagen S, Cromwell O, Becker WM, Fiebig H: **Complementary DNA cloning and expression of a newly recognized high molecular mass allergen Phl p 13 from timothy grass pollen (*Phleum pratense*).** *Clin Exp Allergy* 2000, **30**:324-332.
- This is the first indication of a grass-pollen allergen that is a polygalacturonase.
13. Rantio-Lehtimäki A: **Aerobiology of pollen and pollen allergens.** In *Bioaerosols Handbook*. Edited by Cox CS, Wathes CM. Boca Raton: Lewis; 1995:387-406.
14. Becker WM: **Characterization of allergens from plants.** In *Food Allergy and Intolerances*. Edited by Eisenbrand G *et al.* Weinheim: VCH; 1996:119-129.
15. King TP, Hoffman D, Löwenstein H, Marsh DG, Platts-Mills TAE, Thoma W: **Allergen nomenclature.** *Clin Exp Allergy* 1995, **25**:27-37.
16. Berrens L: **What is a 'major' allergen?** *Clin Exp Allergy* 1994, **24**:606-609.

17. Marsh SGE, Parham P, Barber LD: **HLA polymorphism, peptide-binding motifs and T cell epitopes.** In *The HLA Facts Book*. Edited by Marsh SGE, Parham P, Barber LD. San Diego: Academic Press; 1999:61-72.
 18. Stanley RG, Linskens HF (Eds): *Pollen. Biology – Biochemistry – Management*. Berlin: Springer; 1974.
 19. Grote M: **In situ localization of pollen allergens by immunogold electron microscopy. Allergens at unexpected sites.** *Int Arch Allergy Immunol* 1999, **118**:1-6.
- The author gives a good overview about visualization of allergens within different pollen species and highlights difficulties in *in situ* fixation of highly water-soluble allergenic proteins which can lead to misinterpretation of localization.
20. Knox RB, Heslop-Harrison J: **Pollen-wall proteins: localization and enzymatic activity.** *J Cell Sci* 1970, **6**:1-27.
 21. Howlett BJ, Knox RB, Heslop-Harrison J: **Pollen wall proteins: release of the allergen antigen E from intine and exine sites in the pollen grains of ragweed and Cosmos.** *J Cell Sci* 1973, **13**:603-619.
 22. Knox RB, Heslop-Harrison J, Heslop-Harrison Y: **Pollen-wall proteins: localization and characterization of gametophytic and sporophytic fractions.** *Biol J Linn Soc* 1975, **7**(suppl 1):177-187.
 23. Howlett BJ, Vithanage HIMV, Knox RB: **Immunofluorescent localization of two water-soluble glycoproteins including the major allergen from the pollen of ryegrass *Lolium perenne*.** *Histochem J* 1981, **13**:461-480.
 24. Staff JA, Taylor PE, Smith P, Singh M, Knox RB: **Cellular location of water-soluble, allergenic proteins in rye grass (*Lolium perenne*) pollen using monoclonal and specific IgE antibodies with immunogold probes.** *Histochem J* 1990, **22**:276-290.
 25. Taylor PE, Staff IA, Singh MB, Knox RB: **Localization of the two major allergens in rye-grass pollen using specific monoclonal antibodies and quantitative analysis of immunogold labelling.** *Histochem J* 1994, **26**:392-401.
 26. Behrendt H, Becker WM, Fritzsche C, Sliwa-Tomczok W, Tomczok J, Friedrichs KH, Ring J: **Air pollution and allergy: experimental studies on modulation of allergen release from pollen by air pollutants.** *Int Arch Allergy Immunol* 1997, **113**:69-74.
 27. Behrendt H, Tomczok J, Sliwa-Tomczok W, Kasche A, Ebner von Eschenbach C, Becker WM, Ring J: **Timothy grass (*Phleum pratense* L.) pollen as allergen carriers and initiators of an allergic response.** *Int Arch Allergy Immunol* 1999, **118**:414-418.
 28. Grote M, Dolecek C, van Ree R, Valenta R: **Immunogold electron microscopic localization of timothy grass (*Phleum pratense*) pollen major allergens, Phl p 1 and Phl p V, after anhydrous fixation in acrolein vapor.** *J Histochem Cytochem* 1994, **42**:427-431.
 29. Grobe K, Becker WM, Schlaak M, Petersen A: **Grass group 1 allergens (beta-expansins) are novel, papain-related proteinases.** *Eur J Biochem* 1999, **263**:33-40.
 30. Fischer S, Grote M, Fahlbusch B, Müller WD, Kraft D, Valenta R: **Characterization of Phl p 4, a major timothy grass (*Phleum pratense*) pollen allergen.** *J Allergy Clin Immunol* 1996, **98**:189-198.
 31. Vrtala S, Fischer S, Grote M, Vangelista L, Pastore A, Sperr WR, Valent P, Reichelt R, Kraft D, Valenta R: **Molecular, immunological, and structural characterization of Phl p 6, a major allergen and P-particle-associated protein from Timothy grass (*Phleum pratense*) pollen.** *J Immunol* 1999, **163**:5489-5496.
 32. Behrendt H, Friedrichs K, Kainka-Staenicke E, Darsow U, Becker WM, Tomingas R: **Allergens and pollutants in the air – a complex interaction.** In *New Trends in Allergy III*. Edited by Ring J, Przybilla B. Berlin: Springer; 1991:467-478.
 33. Grote M, Vrtala S, Niederberger V, Valenta R, Reichelt R: **Expulsion of allergen-containing materials from hydrated rye grass (*Lolium perenne*) pollen revealed by using immunogold field emission scanning and transmission electron microscopy.** *J Allergy Clin Immunol* 2000, **105**:1140-1145.
- It is shown that, besides the appearance of allergen-specific immunogold reactivity on the exine's surface, parts of the cytoplasm are extruded through the pores. The experimental set-up was to put pollen into rainwater for two hours. During this time-period, however, physiological tube-formation would already have started. Therefore, the extrusion of cytoplasmic material, including starch granules, during heavy rain is the result of a physiological process that is restricted to pollen.
34. Knox RB: **Grass pollen, thunderstorms and asthma.** *Clin Allergy* 1993, **23**:354-359.
 35. Suphioglu C: **Thunderstorm asthma due to grass pollen.** *Int Arch Allergy Immunol* 1998, **116**:253-260.
 36. Marks GB, Colquhoun JR, Girgis ST, Hjelmroos Koski M, Treloar ABA, Hansen P, Downs SH, Car NG: **Thunderstorm outflows preceding epidemics of asthma during spring and summer.** *Thorax* 2001, **56**:468-471.
- In this carefully controlled case/control study, which was performed in six towns in South-Eastern Australia, a close temporal association between the arrival of the thunderstorm outflow, the rise in the concentration of ruptured pollen grains and the epidemic onset is shown. A mechanism is provided by which thunderstorm outflows confine atmospheric particles in a narrow band of air near ground level.
37. Newson R, Strachan D, Archibald E, Emberlin J, Hardaker P, Collier C: **Acute asthma epidemics, weather and pollen in England, 1987-1994.** *Eur Respir J* 1998, **11**:694-701.
 38. Anto JM, Sunyer J: **Thunderstorms: a risk factor for asthma attacks.** *Thorax* 1997, **52**:669-670.
 39. Spieksma FTM, Kramps JA, van der Linden AC: **Evidence of grass-pollen allergenic activity in the smaller micronic atmospheric aerosol fraction.** *Clin Exp Allergy* 1990, **20**:273-280.
 40. Spieksma FTM, Nikkels BH, Dijkman JH: **Seasonal appearance of grass pollen allergen in natural, pauci-micronic aerosol of various size fractions. Relationship with airborne grass pollen concentration.** *Clin Exp Allergy* 1995, **25**:234-239.
 41. Schäppi GF, Monn C, Wüthrich B, Wanner HU: **Direct determination of allergens in ambient aerosols: methodological aspects.** *Int Arch Allergy Immunol* 1996, **110**:364-370.
 42. Schäppi GF, Suphioglu C, Taylor PE: **Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere.** *J Allergy Clin Immunol* 1997, **100**:656-661.
 43. Schäppi GF, Taylor PE, Pain MCF, Cameron PA, Dent AW, Staff IA, Suphioglu C: **Concentrations of major grass group 5 allergens in pollen grains and atmospheric particles: implications for hay fever and allergic asthma sufferers sensitized to grass pollen allergens.** *Clin Exp Allergy* 1999, **29**:633-641.
 44. Riediker M, Koller T, Monn C: **Differences in size selective aerosol sampling for pollen allergen detection using high-volume cascade impactors.** *Clin Exp Allergy* 2000, **30**:867-873.
 45. Monn C, Koren HS: **Bioaerosols in ambient air particulates: a review and research need.** *Rev Environ Health* 1999, **14**:79-89.
- An excellent review, which highlights current knowledge on bioaerosols and critically discusses all the information so far available about pollen, fungi and spores, and bacteria. Special reference is made to endotoxins and their interaction with ambient particles.
46. Risse U, Tomczok J, Huss-Marp J, Darsow U, Behrendt H: **Health-relevant interaction between airborne particulate matter and aeroallergens (Pollen).** *J Aerosol Sci* 2000, **31**(suppl 1):S27-S28.
 47. von Mutius E, Fritzsche C, Weiland SK, Roll G, Magnussen H: **Prevalence of asthma and allergic disorders among children in united Germany: a descriptive comparison.** *Br Med J* 1992, **305**:1395-1399.
 48. Ring J, Krämer U, Schäfer T, Abeck D, Vieluf D, Behrendt H: **Environmental risk factors for respiratory and skin atopy: results from epidemiologic studies in former East and West Germany.** *Int Arch Allergy Immunol* 1999, **118**:403-407.
 49. Knox RB, Suphioglu C, Taylor P, Desai R, Watson HC, Peng JL, Bursill LA: **Major grass pollen allergen Lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution.** *Clin Exp Allergy* 1997, **27**:246-251.
- This paper provides evidence, for the first time, for a selective binding of an allergen to ambient particles.
50. Behrendt J, Becker WM, Friedrichs KH, Darsow U, Tomingas R: **Interaction between aeroallergens and airborne particulate matter.** *Int Arch Allergy Immunol* 1992, **99**:425-428.
 51. Behrendt H, Kasche A, Ebner von Eschenbach C, Risse U, Huss-Marp J, Ring J: **Secretion of proinflammatory eicosanoid like substances precedes allergen release from pollen grains in the initiation of allergic sensitization.** *Int Arch Allergy Immunol* 2001, **124**:121-125.
- This is the first report about the existence and bioavailability of pollen-derived, proinflammatory, leukotriene-B₄-like substances, with possible biological impact on man.