# Pollen Grains Contain and Release Not Only Allergens, but Also Eicosanoid-Like Substances with Neutrophil Chemotactic Activity: A New Step in the Initiation of Allergic Sensitization?

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## Summary

Pollen grains as allergen carriers are the elicitors of the most common allergic diseases, namely seasonal allergic rhinoconjunctivitis („hay fever"), extrinsic bronchial asthma and other immediate-type allergic diseases.

All of them have increased in prevalence dramatically during the last decades. It is common belief that pollen themselves are inert and act by release of protein allergens in humid conditions on the mucosal surface, where the process of sensitization starts through the recognition of the allergen by an antigen-presenting cell. All studies dealing with the mechanism of this early phase of sensitization have used isolated allergens (from extracts or recombinant technology) as stimulus. However, under natural exposure conditions, the bioavailability of allergen depends upon allergen liberation from internal binding sites within the allergen carrier, here the pollen grain. It is not the soluble allergen, but rather the pollen grain as a particle which comes into contact with the body's surface on the skin or the mucosa. We have shown earlier that the release of allergen from pollen grains can be modulated by external factors such as gaseous or particulate air pollutants.

We now have found that pollen grains from different plants (Timothy grass = *Phleum pratense*, birch = *Betula alba*) secrete significant amounts of eicosanoid-like substances in protein-free buffer solution in a pH, time and temperature-dependent fashion. When pure pollen grains were incubated together with suspensions of human polymorphonuclear leucocytes (PMN), these cells assembled around the pollen grains and showed signs of activation and mediator release leading to destruction of the pollen grain. Leukotriene B4-like activity secreted differed between pollen species with highest values for grass and birch pollen and significantly lower values for pine pollen (*Pinus silvestris*). Furthermore there was a significant modulatory effect from traffic-related pollutants, e.g. volatile organic compounds (VOCs) leading to a significant increase in secretion of LTB4-like activity from pollen grains.

This finding opens a new dimension in understanding the early events in allergic sensitization indicating that pro-inflammatory effects of the allergen carrier itself (the pollen grain) induce activation of cellular constituents of the host. We propose to call this phase the „initiation" of allergic sensitization. The differences in allergenic „potency" of various allergens may be explained by these new findings independent of allergen release from pollen. It also may be helpful in understanding so far unexplained differences in allergy prevalence associated with automobile exhaust exposure.

## Introduction

Allergic diseases (namely allergic rhinoconjunctivitis, bronchial asthma, atopic eczema) have increased in prevalence during the last decades world-wide; the causes for this increase are not known. Among hypothetical concepts under discussion, lack of adequate stimulation of the immune system, improved hygiene, socio-economic factors („life style") as well as influence of environmental pollutants have gained substantial public and scientific attention [1, 3, 6, 10, 11, 14, 16, 18, 22–25].

Allergies are among those few diseases in which environmental factors of both natural and anthropogenic origin have been identified as causal in the disease both in the development of sensitization as well as in the elicitation and aggravation of disease symptoms. We have shown earlier that outdoor air pollution differs strikingly in quality – not only in quantity – between former Eastern and Western European countries, whereby the modern type air pollution – characterized by organic compounds, nitrogen oxides, fine particles and ozone – was associated in multivariate regression analyses with increased prevalence of IgE-mediated sensitization and atopic disease [3, 10, 18]. Pollen grains collected from industrial regions with high polycyclic aromatic hydrocarbon load in West Germany, not in East Germany were shown to be agglomerated with fine airborne particles leading to morphological changes of the pollen surface and altered allergen release [1, 2]. Thus the bioavailability of pollen allergens may be influenced by interaction between pollen and air pollutants in the atmosphere [4]. It is common belief that allergic sensitization starts with the contact between the allergen and the surface of the antigen-presenting cell at the level of the mucosa or the skin. Most studies dealing with this aspect have used allergen extracts as stimulus. Under natural exposure conditions, however, the bioavailability of allergen depends on allergen liberation from internal binding sites within the allergen carrier (e.g. pollen grains) [4, 7, 13, 21]. Here we report the surprising finding that pollen grains themselves liberate substances with pro-inflammatory activity under humid conditions.

### Allergen Liberation from Pollen Grains

Pollen grains are multicellular male gametothytes of both angiosperms and gymnosperms. Anemophilous pollen from gymnosperms (wind-transported) like grass pollen are sealed in a double-layered wall with an outer layer (exine) (containing the lipophilic sporopollenin) and a softer inner layer (intine) enclosing the cytoplasm with subcellular organelles, starch granules and polysaccharide particles [21]. Pollen allergens are bound within the pollen grain around organelles, P-particles and starch granules, some in the cytoplasmic matrix. The liberation of allergens from intracellular binding sites is the prerequisite for allergic sensitization in a pre-disposed individual. The most important stimulus for allergen liberation is humidity [4, 7, 8, 20, 21]; under humid conditions, allergens are rapidly released from pollen grains in pH and temperature-dependent fashion through small caniculi connecting the inner surface of the intine with the outer pollen surface.

Allergen liberation from pollen grains can occur in two different compartments:
- On the surface of the mucosa of the upper respiratory tract
- Outside the individual organism in the ambient air [1, 2]

Allergenic activity has been detected in air samples and fractions below 1 μm in diameter [19, 20].

## Influence of Air Pollutants on Allergen Bioavailability

Pollen collected in polluted atmospheres over West German cities have been found to show particle agglomeration onto the pollen surface (see Fig. 1) as well as signs of local preactivation [1]. Pollen collected at road sides with heavy traffic showed significantly reduced allergen release compared to pollen collected from rural

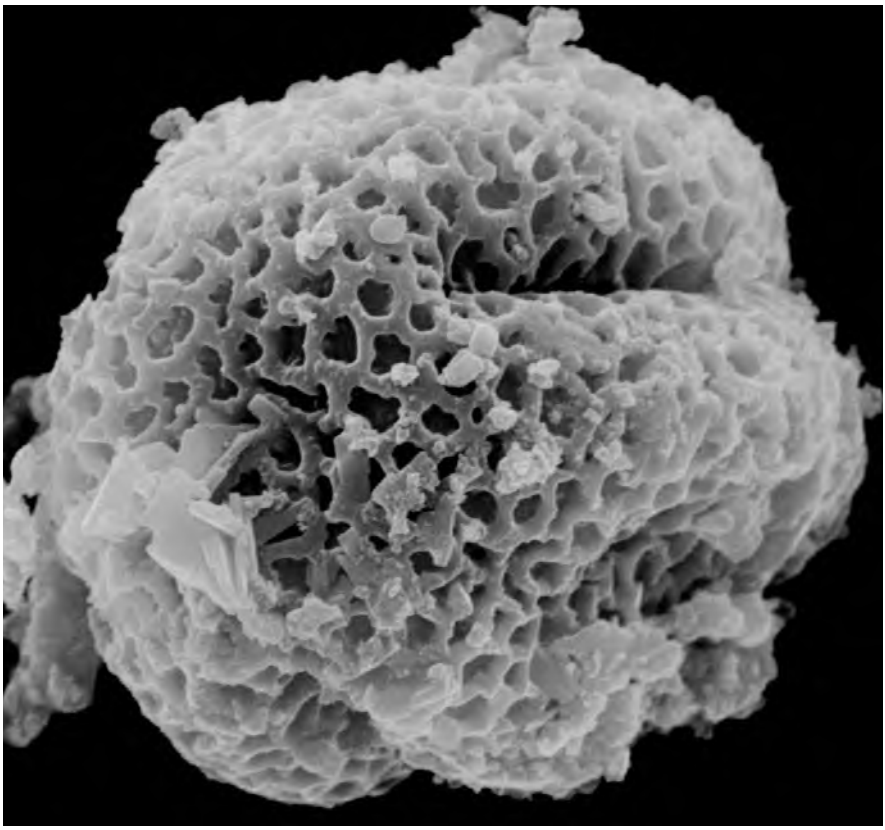

**Fig. 1.** Scanning electron micrograph of a pollen grain from an urban collection site showing particle agglomeration onto pollen surface.

meadows [4]. Since traffic exposure has been found to be associated with high prevalence rates of allergic sensitization and disease [10, 23], this finding might indicate a continuous release of allergen from pollen and binding of free allergen to airborne particles (e.g. diesel exhaust particles).

Using a specially constructed fluidized bed reactor, the influence of both gaseous and particulate air pollutants upon allergen release from pollen has been studied in a dose, time and humidity-dependent fashion [2, 4, 15]. Exposure of pollen to high concentrations of $SO_2$ did not affect allergen release [15]. This observation could help to explain the well-known paradoxic finding of lower asthma and hay fever prevalence rates in children from heavily polluted areas in former East Germany ($SO_2$) compared with children from West Germany (type II air pollution) [3, 12] (see below).

These findings led to the hypothesis that allergen-containing aerosols are generated through pollen-particle interaction in a moist atmosphere.

## Release of Eicosanoid-Like Proinflammatory Mediators from Pollen Grains

Pollen grains incubated in phosphate-buffered saline secreted significant amounts of eicosanoid-like substances as measured by enzyme-immuno assay for leukotriene B4 (30 min at 37°C, pH 6, 7.4 and 9.0).

There were significant differences in total amounts of eicosanoid-like substance released between different pollen species: The highest values were found from birch pollen, grass and mugwort pollen, whereas pine pollen showed only little LTB4-like immunoreactivity [5].

When pollen grains were brought into contact with human neutrophil granulocytes, neutrophils were attracted and bound firmly to the pollen surface showing signs of activation and release of neutrophil mediators (C. Traidl, in prep.).

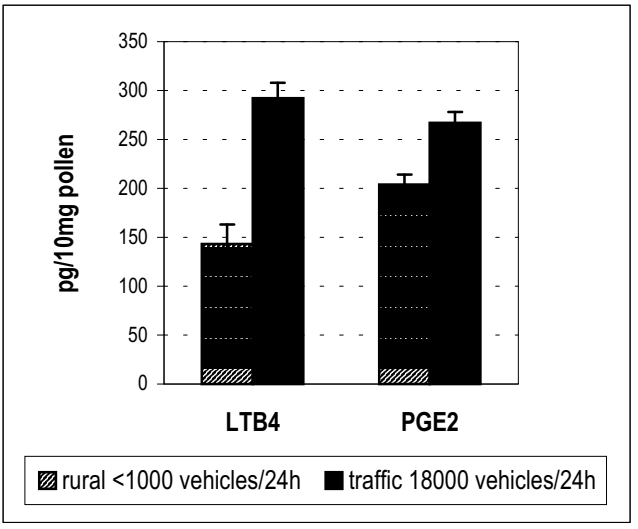


**Fig. 2.** Enhanced release of eicosanoid-like substances from *P. pratense* pollen freshly collected from traffic-exposed area compared to a rural meadow [5]

Similar findings were observed for the interaction between pollen grains and eosinophil granulocytes (S. Plötz, in prep.).

There were significant differences between various pollen grain sources: Pollen freshly collected from pollinating *Phleum pratense* (Timothy grass) growing on a rural meadow in upper Bavaria with a car traffic below 1,000 vehicles/24 hours showed a significantly reduced release of pro-inflammatory eicosanoid-like substances compared to pollen freshly collected and growing on the roadside of a high-traffic road in Southern Munich (car traffic more 18,000 vehicles/24 hours) (Fig. 2).

In the fluidized bed reactor, pollen grains were exposed to different types of air pollutants under various degrees of relative humidity. There was a marked enhancement of proinflammatory $LTB_4$-like substances by exposure to volatile organic compounds (characteristic for type II air pollution) [15].

## Conclusions

These findings which have to be further studied in detail have wide implications; they open a new dimension in the understanding of the early events in allergic sensitization indicating that pro-inflammatory effects produced by the allergen carrier itself (namely the pollen grain) contribute to the activation of cellular constituents of the host, as e.g. neutrophil granulocytes or eosinophils. It is especially remarkable that pollen grains from plants with high allergenicity (birch grass, mugwort) produce significantly higher amounts of these pro-inflammatory substances than pine pollen with a much less pronounced allergenicity. Thus, the term „allergenic potency" might have to be re-defined; it should not only be considered at the molecular level of the protein structure of the allergen, but maybe also include the characteristics of allergen release and bioavailability from its carrier. The process of initiation of sensitization precedes the contact between allergen and antigen-presenting cell and may be the very first step in atopic sensitization, later followed by T-cell activation, antibody production and development of immunological memory as well as recruitment of amplificatory cells.

The aspect of allergen bioavailability should be considered more thoroughly in the future. Unfortunately, until today, only little data are available regarding a quantitative dose-response relationship for outdoor allergen exposure and allergic sensitization or disease.

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