International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2001;124:121–125

Secretion of Proinflammatory Eicosanoid-Like Substances Precedes Allergen Release from Pollen Grains in the Initiation of Allergic Sensitization

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Key Words
Pollen · Eicosanoid-like substances · Air pollution · Allergy

Abstract
It is commonly believed that allergic sensitization starts when an allergen contacts the surface of an antigen-presenting cell in mucosal or skin epithelia. Most studies dealing with this aspect use 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. In comparing total protein and major allergen release from timothy grass (*Phleum pratense* L.) pollen freshly collected on rural meadows or near high-traffic roads, there was a striking difference between the pollen, with higher allergen release rates from rural meadow pollen grains. Thus, allergen release does not explain the higher prevalence rates of atopic sensitization and disease observed in many epidemiological studies in children exposed to automobile exhaust. Therefore, other possible effectors from pollen grains were investigated. Pollen grains incubated in protein-free buffer were found to secrete significant amounts of eicosanoid-like substances, namely leukotriene (LT) B_4-like and prostaglandin E_2-like substances, in a pH-, time- and temperature-dependent fashion. The highest values of eicosanoid secretion were found in birch, grass and mugwort pollen, while pine (*Pinus sylvestris* L.) pollen showed only marginal eicosanoid-like secretion. Additionally, the release of these substances was significantly higher from pollen which had been collected near roads with heavy traffic, indicating a stronger proinflammatory activity of these pollen grains. In order to investigate the effects of air pollutants, native pollen grains were exposed in a dose- and time-dependent fashion in a fluidized bed reactor to traffic-related pollutants, e.g. volatile organic compounds (toluene, m-xylene), leading again to a significant increase in the secretion of LTB_4-like immunoreactivity, in contrast to exposure with sulfur dioxide. This finding opens a new dimension of understanding of the early events in allergic sensitization, indicating that proinflammatory effects of the allergen carrier, e.g. the pollen grain itself, can lead to activation of the mucosal membrane. These findings might help to also explain the higher prevalence rates of pollen allergy in areas with high automobile exhaust emissions. Fur-

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1018–2438/01/1243–0121$17.50/0

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thermore, the allergenic 'potency' of various allergens has to be redefined at the allergen carrier level with regard to different stages of allergen and mediator release prior to the contact with the host's immune system.

Introduction

The fact that atopic diseases (allergic rhinoconjunctivitis, bronchial asthma, atopic eczema) have increased in prevalence during the last decades is a generally accepted and worldwide phenomenon [1–4]. The causes for this increase are not known. Among many hypotheses, the idea that environmental pollutants may play a role has gained substantial public and scientific attention [5–8]. Previously, we have shown that outdoor air pollution differs strikingly in quality – not only quantity – between Eastern and Western European countries, whereby the modern type II air pollution was characterized mostly by organic compounds, nitrogen oxides, fine particles and ozone [6]. Type II pollution was associated in multivariate regression analysis with increased prevalence rates of IgE-mediated sensitization and atopic disease [9]. Pollen grains collected from industrial regions with high polycyclic aromatic hydrocarbon load in West Germany, but not in East Germany, were shown to be agglomerated with fine airborne particles, leading to morphological changes to the pollen surface and altered allergen release [10, 11]. Thus, the bioavailability of pollen allergens may be influenced by air pollutants. These data have supported the concept of an interaction between pollen and pollutants in the atmosphere outside of the organism [5, 12, 13] and emphasize the importance of dose-response relationships with regard to allergen exposure and allergic disease in the outdoor environment [11].

It is commonly believed that allergic sensitization starts with the contact between the allergen and the surface of the antigen-presenting cell in the mucosa or the skin. Most studies dealing with this aspect use 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 [11]. In studying the interaction of pollen grains with inflammatory cells, we made the surprising finding that, apart from allergen, pollen grains liberate substances with proinflammatory activity under humid conditions [11]. Here, we studied the nature and effect of this proinflammatory activity from pollen grains of different species as well as the influence of outdoor air pollutants upon the liberation of pollen substances with relevance to allergy.

Materials and Methods

Pollen Grain Sources

The pollen grains studied were purchased from commercial producers (Allergon, Sweden; Sigma, Germany). Pollen was also freshly collected from pollinating *Phleum pratense* (timothy grass) plants growing either on a rural meadow in upper Bavaria (car traffic below 1,000 vehicles per 24 h) or on the roadside on a high-traffic road in southern Munich (car traffic more than 18,000 vehicles per 24 h).

Allergen and Eicosanoid-Like Substance Release

Pollen grains were incubated in vitro in phosphate-buffered saline (PBS; 30 min at 37°C at pH 6.0, 7.4 and 9.0). The concentrations of the eicosanoid-like substances were measured by commercially available enzyme immunoassays for prostaglandin (PG) E_2 and leukotriene (LT) B_4 (Amersham Pharmacia, Germany). Eicosanoid-like substances were further identified by HPLC.

In vitro Pollen Grain Exposure to Air Pollutants

In order to investigate the effects of air pollutants on pollen, pollen grains were incubated in a fluidized bed reactor under controlled conditions of relative air humidity with traffic-related pollutants such as volatile organic compounds (toluene, m-xylene), as well as the classical pollutant sulfur dioxide, as described previously [14].

Statistical Analysis

If not mentioned otherwise, mean values and standard deviations (SD) are given. The statistical significance of differences was calculated using the Student's t test.

Results

Release of Eicosanoid-Like Substances from Pollen Grains

Pollen grains incubated in PBS secreted significant amounts of eicosanoid-like substances, as measured by enzyme immunoassays for PGE_2 and LTB_4. There were significant differences regarding the total amounts of eicosanoid-like substances released from different pollen species, with the highest values for PGE_2-like immunoreactivity from birch pollen and very little LTB_4-like immunoreactivity released from pine (*Pinus sylvestris* L.) pollen (fig. 1). The release of PGE_2- and LTB_4-like immunoreactivity from pollen grains was pH, time and temperature dependent (fig. 2).

PGE_2- and LTB_4-like immunoreactivity was further characterized by HPLC, with peaks very closely related to PGE_2 and LTB_4, respectively.

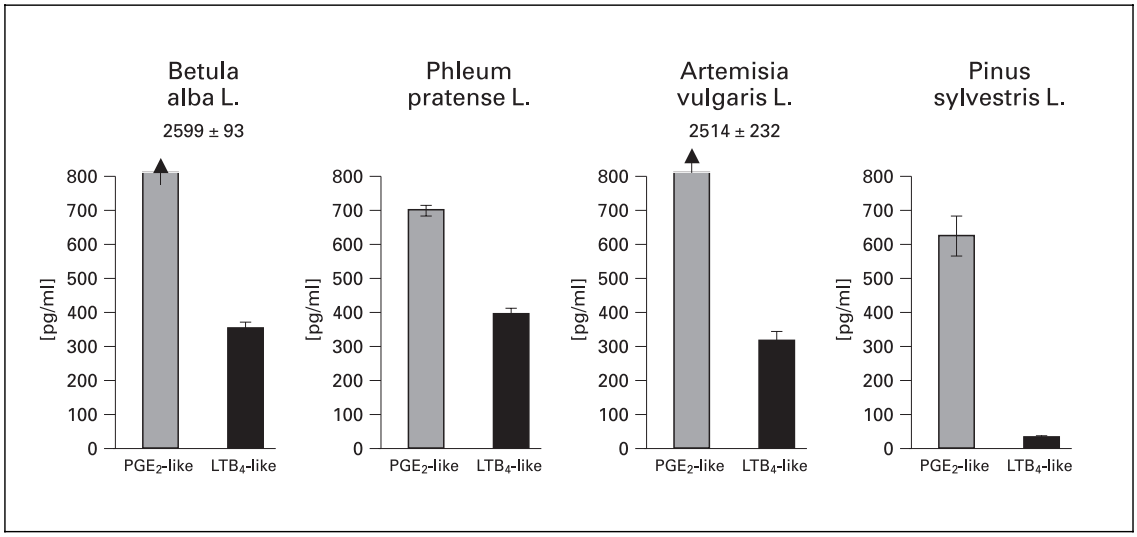

Fig. 1. In vitro release of eicosanoid-like substances from birch (*Betula alba* L.), grass (*P. pratense* L.), mugwort (*Artemisia vulgaris* L.) and pine (*P. sylvestris* L.) pollen into aqueous milieu (10 mg pollen/ml PBS, pH 7.4, 37°C; mean ± SD, n = 8). Note that nonallergenic pine pollen did not release substantial amounts of proinflammatory eicosanoid-like substances.

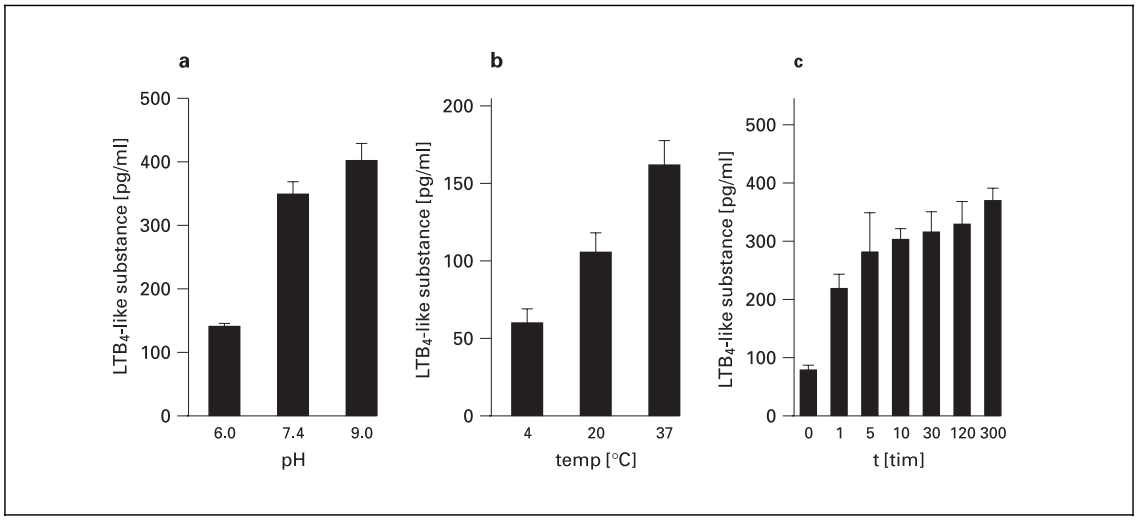

Fig. 2. Release of eicosanoid-like substances from birch pollen as a function of pH (**a**), temperature (**b**) and time (**c**).

Influence of Air Pollutants upon Pollen Grains

In control experiments using synthetic air as the exposure medium and various degrees of relative humidity, the effect of typical air pollutants for type I (sulfur dioxide) or type II (toluene, m-xylene) air pollution was studied in vitro in a fluidized bed reactor using timothy grass pollen. While sulfur dioxide significantly inhibited the release of proinflammatory eicosanoid-like substances from pollen grains, there was a significant enhancement by exposure to volatile organic compounds at environmentally relevant concentrations.

In addition, pollen grains which had been collected from two sites with different air pollution displayed the release of substantially higher amounts of proinflammatory eicosanoid-like substances when exposed to traffic exhaust (fig. 3).

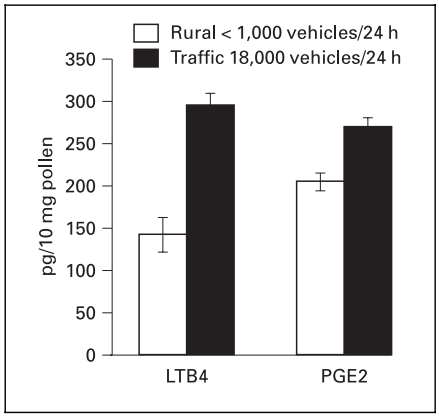

Fig. 3. Enhanced release of eicosanoid-like substances from *P. pratense* pollen freshly collected from a high-traffic area as compared to a rural meadow, indicating stronger proinflammatory activity.

Discussion

The results of this study show that pollen grains by themselves are able to liberate proinflammatory activity measured as eicosanoid-like substances under humid conditions without contact with the organism or its inflammatory cells. Thus, together with allergen exposure on the mucosal surface, proinflammatory substances are released from pollen and are able to activate human inflammatory cells, as has been shown using polymorphonuclear leukocytes [15]. In fact, agglomeration of human neutrophils around pollen grains has been observed previously and may be mediated by transferrin [16].

This finding opens up a new dimension in the understanding of the early events in allergic sensitization, which we would like to call 'initiation of sensitization'. The allergen carrier, e.g. the pollen grain itself, can contribute to activation of the mucosal membrane by proinflammatory activity. It is especially remarkable that pollen grains from plants with high allergenicity (birch, grass, mugwort) produce significantly higher amounts of these proinflammatory substances than pine pollen, which exhibits much less pronounced allergenicity. So, the term 'allergenic potency' in relation to various allergens is not only to be considered at the molecular level of primary, secondary and tertiary protein structure, but should also include by definition the ability to enhance sensitization by directly activating inflammatory cells from the organism. Candidates (i.e. polymorphonuclear leukocytes) are usually found on the surface of the nasal mucosa in normal healthy volunteers as well as in atopics [17].

The fact that different types of air pollutants exert opposite effects upon the release of eicosanoid-like substances from pollen grains, namely an inhibiting effect by SO_2 and an enhancing effect by volatile organic compounds, might help to explain the higher prevalence rates of pollen sensitization and allergy in areas with high levels of automobile exhaust emission [6, 18, 19].

We speculate that a process of initiation of allergy precedes the allergen/antigen-presenting cell interaction and may be the very first step in atopic sensitization prior to antigen presentation, T cell activation, antibody production and the development of immunological memory as well as recruitment of amplificatory cells.

The results of this study might also help to further explain the well-known and so far not perfectly understood differences in allergy prevalence between Eastern and Western European populations apart from factors of lifestyle or hygiene [20, 21].

Acknowledgment

This study was supported by a grant from the Bavarian Ministry of Environment (BStMLU) and a grant from the Federal Ministry of Research and Education (BMBF) 'Klinische Forschergruppe Molecular and Clinical Allergotoxicology'.

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