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Air Pollution and Allergy: Experimental Studies on Modulation of Allergen Release from Pollen by Air Pollutants

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Abstract

The fact that allergic diseases increase in prevalence is a generally accepted and worldwide phenomenon. The causes for this increase are not known: only hypothetical concepts exist. Epidemiological studies comparing Eastern and Western European populations have shown a striking difference in the prevalence of respiratory atopic diseases, which is lower in the East. At the same time, different patterns of air pollution have been described, namely 'classical' type I, characterized by SO₂ and dust prevailing in the East, and 'modern' type II, characterized by organic compounds, fine particles and ozone, which is more prominent in the West. Type II was associated in multivariate regression analysis with increased prevalence of IgE-mediated allergy. Pollen grains collected from industrial regions with high polyaromatic hydrocarbon load in West Germany, but not in East Germany, were shown to be agglomerated with airborne particles. In vitro exposure of pollen to particles indicated morphological changes and increased allergen release from the pollen. In vitro exposure of pollen to gaseous pollutants (SO₂ and NO₂) under different conditions of humidity resulted in SO₂-induced, but not NO₂-induced reduction of allergen release from pollen. It is concluded that the bioavailability of grass pollen allergens may be modulated by air pollutants, supporting the concept of an interaction between pollen and pollutants in the atmosphere outside the organism which in turn may affect allergy-relevant phenomena.

Key Words

Air pollution
Allergy
Pollen
Allergens
Pollen-pollutant interaction
Particles
Sulfur dioxide
Exposure chamber

Introduction and Epidemiology

The prevalence of allergic diseases has increased worldwide during the last decades [1–4]. The reasons for this increase are unknown. Among many hypotheses, the idea that environmental pollutants may play a role has gained substantial public and scientific attention [5–8]. In order to study dose-response relationships between disease outcome and exposure measurements and to monitor the effects of air

pollutants on human health with special reference to allergic diseases, several population-based trials have been performed by our group in the reunited Germany [9–13]. These studies included more than 20,000 preschool children, aged 5–6 years, living in different areas of East and West Germany with different type and intensity of air pollution. One of the most surprising results of these epidemiological investigations was the fact that respiratory atopic diseases were strikingly less frequent in the East than in the West [10–12].

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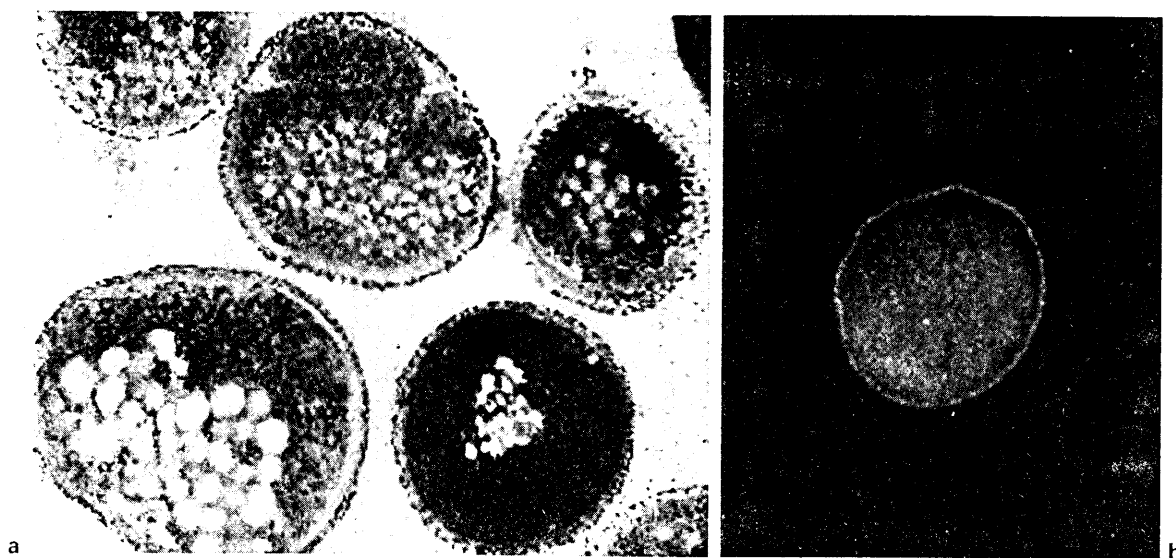


Fig. 1. Localization of major grass pollen allergen Phl p 5 within the pollen after strictly anhydrous fixation and embedding procedures. Staining of semithin sections (**a**) and cryosections (**b**) with monoclonal antibodies (mAb Bo 1) and visualizing by silver enhanced immunogold (**a**) and by canine-anti-mouse FITC conjugate. Light micrographs. **a** $\times 1250$, **b** $\times 312$.

Similar findings have been reported by other investigators [14]. Since air pollution (namely particles and sulfur dioxide) has been a characteristic feature of former communist countries in Eastern Europe, many authors concluded from these data that air pollution does not play any role in the development of allergy. However, from our investigations it has become clear that different patterns of outdoor air pollution have diverse effects on allergic diseases [15, 16]. Multivariate regression analysis of our data revealed a significant association between air pollutants emitted from automobile exhaust and indoor gas sources – type II pollutants [15, 16] – and parameters of allergic sensitization in West Germany after controlling for other covariates such as smoking habits, family history of atopy, age, sex, socioeconomic status [12, 15]. On the other hand, the prevalence of infectious and irritant responses of the upper respiratory tract was clearly correlated with exposure to and intensity of the air pollutants dust and sulfur dioxide – type I pollutants [15]. These health outcome parameters significantly decreased in prevalence with the decrease in pollutant concentration from 1991 to 1994 [13].

So far, the differences in the prevalence of allergy between Eastern and Western European countries cannot be explained. Hypothetical considerations involve the 'lifestyle', which was clearly different between East and West [17], but not very scientifically defined.

The Concept of Pollen-Pollutant Interaction

Pollen grains are the major source of outdoor aeroallergens. Independently of pollutants and other environmental factors, exposure to allergen is the primary condition for predisposed individuals to become sensitized, as is also true for development of disease and for elicitation of symptoms of allergic inflammation. In the natural exposure situation, the bioavailability of allergens depends on allergen removal from internal pollen-binding sites (fig. 1) and on substantial release into the external milieu, in order to provoke a reaction. In principle, this allergen release can occur at two different compartment sites: (1) on the mucous respiratory tract surface of sensitized (and nonsensitized) individuals after pollen inhalation and (2) outside the individuals in the ambient air itself. Both events, however, probably require a moist atmosphere which then in turn affects the amount of allergen liberated at the site of reaction. Allergenic activity has indeed been detected by several groups in air sample fractions below $1\text{ }\mu\text{m}$ in diameter [19, 20].

Therefore, in the outdoor environment, two different allergen-containing compartments exist in the ambient air: the pollen itself and the allergenic aerosol, a fact that may explain why – unlike the situation in the indoor environment – the risk of sensitization against outdoor allergens cannot be adequately estimated by simple pollen quantification.

On the other hand, evidence is available for an interaction between aeroallergens and pollutants at the level of either plant growth and pollen maturation or of airborne pollen which had been emitted into a polluted atmosphere and which are then exposed to the contaminants (fig. 2). Therefore, in polluted areas the interaction between pollen and pollutants, resulting in quantitative changes and qualitative alterations of aeroallergens, seems to be a common feature.

We have shown earlier [18] that pollen collected over highly polluted industrial regions in West Germany show intense agglomeration with airborne particles similar to pollen collected near roads with heavy traffic [21]. Moreover, semiquantitative analysis indicates that the amount of particle agglomeration is positively associated with the particle-bound aromatic hydrocarbon load in the air [18], as is true for particulate metal concentrations, which are different from city to city due to their industrial nature [22, 23].

Influence of Particulate Pollutants: In vitro Studies on Allergen Release

In vitro studies on the effect of extracts of airborne particles on pollen morphology showed characteristic ultrastructural changes at the surface of grass pollen and within the protoplasm, such as exocytosis of granular proteinaceous material [18]. At the same time, a significant increase of protein release from pollen was demonstrated dose and time dependently (fig. 3), indicating the direct action of substances adsorbed to airborne particles on structural elements of the pollen. Using SDS polyacrylamide gel electrophoresis immunoblotting and probing the blots with various monoclonal antibodies as well as with serum of pollen allergic patients, an alteration of allergenic protein bands – increase in Phl p 1 intensity, dose-dependent loss of Phl p 6, new bands at 60 kD, shift of binding intensity of IgE reactive bands to the acidic site – can be demonstrated (fig. 4). In 2D electrophoresis after probing the blot with monoclonal antibodies recognizing Phl p 5 there was evidence for the appearance of acidic isoforms [manuscript in preparation].

From these data it can be concluded that substances adsorbed to airborne particles can mediate agglomeration of particles onto pollen surfaces followed by preactivation of pollen and then, under appropriate conditions (humidity etc.), may induce circumscribed local allergen release. One has therefore to take into consideration that in regions with high air pollution, particles are not only carriers of pollutants but also of allergens and, as has been pointed out earlier [15, 18, 21], that pollen grains are not only carriers of allergens but also of pollutants.

POLLEN ↔ POLLUTANT

1. Plant growth and pollen maturation

incorporation of heavy metals
sulphur
others



pollen counts

2. Pollen in the atmosphere

> climate factors
> air humidity
> pollutants [APM, gases]



qualitative changes of pollen

vitality
morphology

modification of allergens
modification of allergen release

generation of aerosols

Fig. 2. Pollen-pollutant interactions.

Influence of Gaseous Air Pollutants: Exposure Studies in a Fluidized Bed Reactor

In order to investigate the effects of gaseous air pollutants, we have constructed a chamber for exposure of pollen to gaseous pollutants in a dose- and time-dependent fashion and under variable conditions of relative air humidity [24, 25] (fig. 5). After exposure, pollen was allowed to release proteins into an aqueous milieu and the samples were then blotted and stained with monoclonal antibodies or with IgE-containing serum.

In control experiments using synthetic air as exposure medium at various degrees of relative humidity, light microscopy and scanning electron microscopy did not reveal

Fig. 3. In vitro effect of aqueous extract of airborne particles (Gex) on protein release from grass pollen demonstrating increased protein release by the pollutant. Control samples were incubated with water. Gex dilution 1:100 corresponds to 6.57 m³ air volume and 0.39 mg dust.

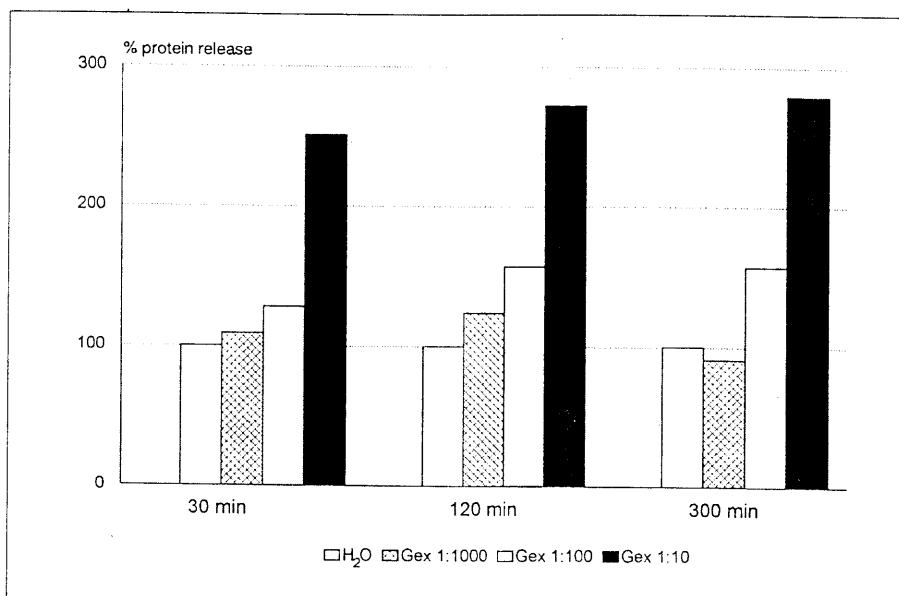
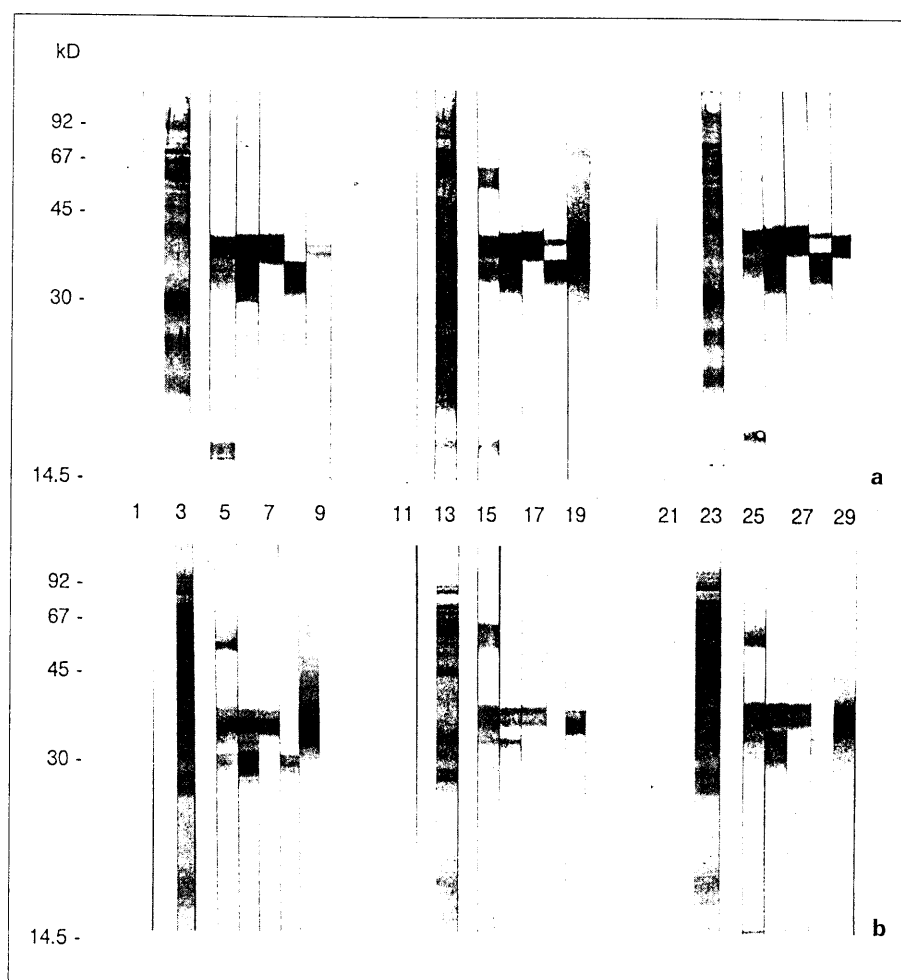


Fig. 4. a Incubation of *P. pratense* pollen with aqua bidest. (lanes 1–9), aqueous Gex (1:10) (lanes 11–19) and aqueous Gex (1:100) (lanes 21–29); 30 min, 37°C. **b** Ammonium carbonate extract of the same timothy grass pollen after incubation with aqua bidest. (lanes 1–9), Gex (1:10) (lanes 11–19) and Gex (1:100) (lanes 21–29); 30 min, 37°C. Lanes 1, 11, 21: anti-mouse control; lanes 2, 12, 22: anti-human control; lanes 3, 13, 23: protein staining; lanes 4, 14, 24: anti-IgE normal serum; lanes 5, 15, 25: anti-IgE patient's serum; lanes 6, 16, 26: mAb Bo 1 (Phl p 5); lanes 7, 17, 27: mAb BG6 (Phl p 5a); lanes 8, 18, 28: mAb Bo9 (Phl p 5b); lanes 9, 19, 29: mAb IG12 (Phl p1).



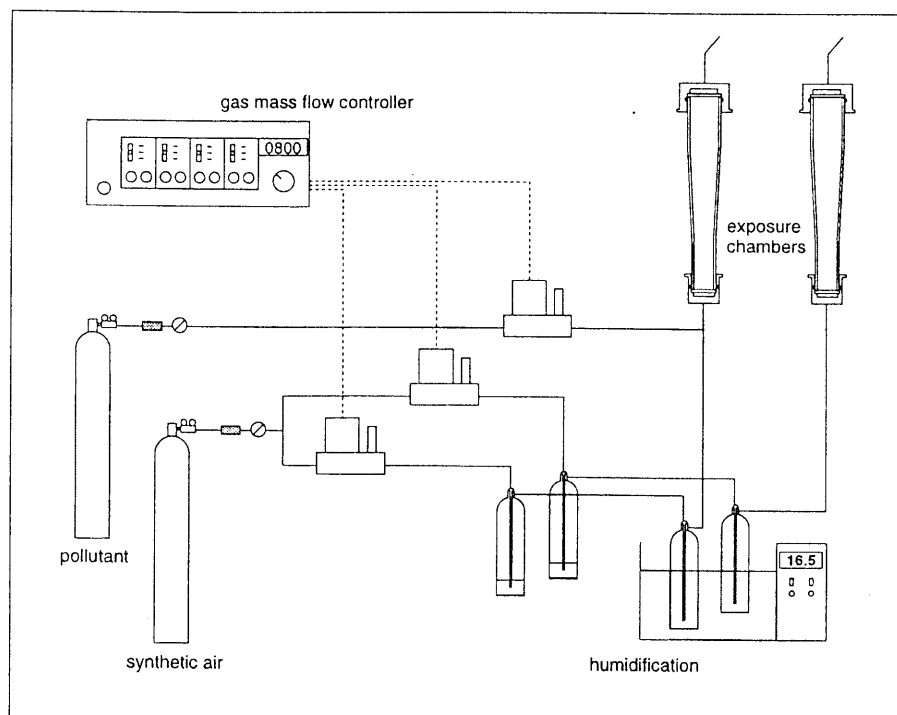


Fig. 5. Schematic diagram of the fluidized bed reactor chamber for exposure experiments of pollen with gaseous pollutants.

any change in pollen structure or vitality for exposure times up to 9 h. Regarding protein release from pollen, there was a significant influence of relative air humidity which has to be controlled in specific experiments [25]. In addition, immunoblots probing with monoclonal antibodies detecting major allergens Phl p 1 and Phl p 5 displayed a reduction of Phl p 1 intensity, but not of Phl p 5 (fig. 6a), over time and with increasing relative humidity [26].

After exposure of *Phleum pratense* pollen to sulfur dioxide, but not to nitrogen dioxide, in a dose- and time-dependent manner, the staining intensity of major allergen Phl p 5 in the supernatants was significantly reduced with time, when exposure involved high concentrations (fig. 6). The SO_2 -induced reduction of Phl p 5 availability in pollen supernatants was further confirmed by quantifying this allergen using ELISA techniques [26].

From these experiments, we speculate that the bioavailability of major grass pollen allergens is grossly reduced in regions polluted with sulfur dioxide as compared to non-polluted regions.

Conclusions

We have shown that allergotoxicology, i.e. the study of the influence of toxic substances upon development, elicitation and persistence of allergic reactions [16], does not

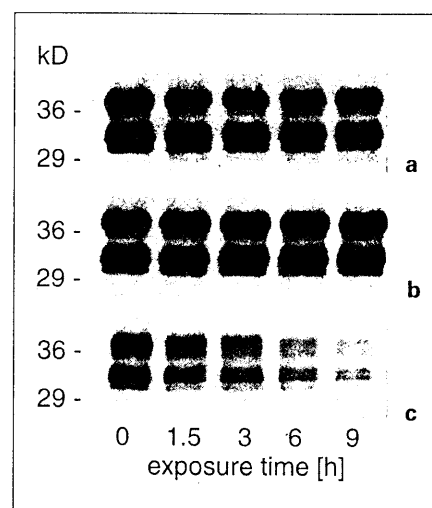


Fig. 6. Immunoblots of aqueous extracts from timothy grass pollen which had been exposed for 0, 1.5, 3, 6, and 9 h to synthetic air (a), 1 mg/m³ NO_2 (b), and 16.5 mg/m³ SO_2 (c) at a relative humidity of 80%. Blots were stained with mAb Bo1 recognizing major allergen Phl p 5. Note the loss of staining intensity in c, but not in b and a.

confine itself to the investigation of organisms, organs or cells but also has to take into account the exposure to environmental substances.

The findings that (1) exposure to particles increases the release of altered allergens and that (2) exposure to the classical air pollutant sulfur dioxide leads to a reduced bioavailability of grass pollen allergen are of interest in explaining the observed differences of allergological parameters in studies of environmental epidemiology.

Summarizing the effects of pollen-pollutant interaction know so far, it has been shown that (1) air pollutants can

change pollen in situ and in vitro with respect to morphology and to allergen release both quantitatively and qualitatively. (2) Pollen – pollutant interaction may therefore affect allergen-relevant phenomena.

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