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Organic Extracts of Urban Aerosol (≤PM2.5) Enhance rBet v 1-Induced Upregulation of CD63 in Basophils from Birch Pollen–Allergic Individuals

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Epidemiological studies have linked the high prevalence rates of IgE-mediated allergic diseases to an increase in exposure to traffic-related air pollutants such as diesel exhaust particles (DEPs). There is growing experimental evidence that organic compounds of DEPs, predominantly polycyclic aromatic hydrocarbons (PAHs), participate in the development and maintenance of allergic airway diseases. In this study we investigated the impact of organic extracts of urban aerosol (AERex) containing various PAH concentrations on the activation of human basophils. Whole blood samples from six birch pollen–allergic and five control subjects were repeatedly incubated in the presence of AERex with or without recombinant Bet v 1 (rBet v 1). Basophils were analyzed for CD63 expression as a measure of basophil activation by using multiparameter flow cytometry. Basophils, when exposed *in vitro* to AERex and rBet v 1, expressed CD63 significantly more than with antigen activation alone. AERex synergized with rBet v 1 in a dose-dependent manner, but did not activate basophils from nonallergic donors. AERex effect on CD63 upregulation was found in blood samples of all patients and did not occur in the absence of rBet v 1. Strongest basophil activation was monitored upon stimulation with AERex comprising the highest PAH content. The capability of AERex to increase activation of basophils from birch pollen–allergic subjects at ambient concentrations suggests an important role of organic compounds of airborne particles in the aggravation of IgE-mediated allergic diseases. This could be a new aspect of regulation of unspecific promoting stimuli in clinical manifestation of allergic inflammation.

Key Words: **basophils; CD63 expression; organic compounds of urban aerosol; PM2.5, IgE-mediated allergic diseases.**

Allergic diseases represent a major environmental health problem. The prevalence of atopic diseases has increased especially in the western hemisphere within the last decades (ISAAC, 1998; Ring, 1997; Ring *et al*., 2001; Saxon and Diaz-Sanchez, 2005; Wüthrich, 1989). One factor for the development of this multicausal process is the allergy-promoting role of anthropogenic air pollutants. Epidemiological studies have demonstrated an association between exposure to atmospheric pollutants, i.e., motor vehicle emissions, and increased symptoms of asthma and allergic rhinoconjunctivitis (Bascom *et al*., 1991; Corbo *et al*., 1993; Heinrich and Wichmann, 2004; Krämer *et al*., 2000; Peterson and Saxon, 1996; Saxon and Diaz-Sanchez, 2005). In particular, the effect of diesel exhaust particles (DEPs) on the pathophysiology of allergic airway disease has been extensively analyzed in both animals and humans subjects by using *in vitro* and *in vivo* systems (Fahy *et al*., 1999; Fujieda *et al*., 1998; Nightingale *et al*., 2000; Salvi *et al*., 1999; Takano *et al*., 1997). These data have clearly identified a relationship between DEP challenge and initiation of an IgE-dependent allergic inflammatory response. However, less attention has been attributed so far to the involvement of organic compounds of diesel emissions in IgE-mediated allergic inflammation at the level of mediator cells. There is growing evidence that organic substances adsorbed to DEPs have an effect on allergen-induced degranulation of mast cells (Diaz-Sanchez *et al*., 2000) and on spontaneous histamine release from and cytokine production in basophilic granulocytes (Devouassoux *et al*., 2002).

The aim of the present study is to characterize the impact of organic extracts of urban particulate matter on human basophils from birch pollen–allergic and nonatopic, healthy subjects and to identify possible synergistic effects of particle-associated organic compounds and Bet v 1, the major allergen

The authors certify that all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration. The ethical committee of the Technical University of Munich approved the study, and volunteers were enrolled in the study after giving written informed consent. The investigation was conducted according to the Declaration of Helsinki.

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of birch pollen grains, upon basophil stimulation from sensitized individuals.

MATERIALS AND METHODS

Sampling of airborne particulate matter (PM). Atmospheric fine dust was collected using an Andersen High-Volume Sampler (Andersen Instruments Inc., U.S.A.) equipped with a 2.5-µm head. Particles were collected on a quartz-fibre filter (GF 20, 203 × 254 mm, Schleicher & Schuell, Germany) which was cleaned by heating at 500°C over 3 h before use. Air was drawn through the filter at an average flow rate of 52 m^3/h. An organic extract of urban aerosol, 1-day sample ($AERex^{1d}$), was collected on February 20–21, 2003 (23.93 h, total air volume 1270 m^3), and a 5-day sample ($AERex^{5d}$) between June 5 and June 10, 2003 (119.89 h, total air volume 6230 m^3). The sampler was located near (<10 m) a highly frequented six-lane road (Ingolstädter Landstrasse: 44,000 motor vehicles/day; personal communication ''Planungsreferat München'') at the main entrance of the GSF research campus in the north of Munich, Germany. The campus has a wide lawn with some trees (no birch trees). The GSF boiler house is 800 m away from the sampling site. Sampling was performed 2 m above ground.

Preparation of PM extracts. One-third of the 1-day filter and two-thirds of the 5-day filter were extracted by steam distillation. Each filter was placed into a flask with 250 ml water. The flask was heated for 2 h at 100°C. At this temperature the majority of allergens are destroyed by denaturation, including Bet v 1. The steam was cooled down by passing through a Liebig cooler, and the aqueous condensate was online extracted by dropping through 2 ml n-hexane. After 2 h the process was stopped, and the n-hexane phase was removed. In order to compare both extracts, 0.1 ml dimethyl-sulfoxide (DMSO) for $AERex^{1d}$ and 1 ml DMSO for $AERex^{5d}$ were added. The n-hexane was vaporized under a gentle stream of nitrogen at 40°C, yielding two organic extracts with similar concentrations of air equivalents per ml.

GC-MS analysis. Identification of compounds was performed using a Hewlett-Packard gas chromatograph–mass spectrometer (GC-MS) system (GC 6890 series, with a 5973N mass selective detector). The MS was operated in the full scan mode (m/z 35–400) with electron impact ionization. The chromatographic separation was accomplished using a 30.0 m DB-5ms column with a 0.25 mm id and 0.25 µm film thickness (J&W Scientific, Rancho, Cordova, CA). Injection was performed in splitless mode. The oven temperature was ramped by 10K min^{-1} from 55°C to 300°C and held for 15 min. The identification of individual compounds was based on the comparison of their retention times and mass spectra to those of pure standard substances.

HPLC analysis. To investigate the variance of polycyclic aromatic hydrocarbon (PAH) composition in $AERex^{1d}$ (1-day sampling in winter) and $AERex^{5d}$ (5-day sampling in summer), a quantitative high pressure liquid chromatography (HPLC) analysis was carried out. An HPLC system, HP 1100 (Agilent Technologies, Böblingen, Germany), equipped with a diode array and a fluorescence detector was used. The analytical column (MZ-PAH C-18, 5 µm, 250 mm, 3 mm id) was purchased from MZ Analysentechnik, Mainz, Germany. The PAHs were separated with an acetonitrile gradient at 308K and 0.5 ml/min; separation started with 58% acetonitrile, and the organic solvent content was increased to 100% in 35 min and finally held at 100% acetonitrile for the last 12 min. Within the next 2 min, the initial eluent composition was reached, and the system was equilibrated for 15 min. Injection volume was 10 µl. Detection and quantification of the PAHs was carried out, applying time-programmed fluorescence detection. The HPLC method was externally calibrated for the quantification of 18 PAHs—15 of them were U.S. EPA priority pollutants—in the range from 2 to 1000 pg/µl using chromatographic peak areas versus concentration. Resulting correlation coefficients of the calibration curves ($n = 6$) were all higher than 0.999.

Determination of LPS in AERex. Lipopolysaccharide (LPS) was quantified by Limulus Amebocyte Lysate (LAL) assay (Cambrex Bio Science, Apen, Germany). AERex samples were provided in DMSO and tested in a 1:100 dilution in LPS-free water. DMSO 1% vehicle did not show any interference with the LAL test system. *E. coli* standard served as a positive control. The technical assistance of Mrs. Britta Dorn is acknowledged.

Subjects. Six atopic individuals (four females, two males, 21–39 years old, mean age 28.5 ± 7.9 years) with known birch pollen allergy and five healthy, nonatopic controls (three females, two males, 23–32 years old, mean age 27.2 ± 4.1 years) were asked to give blood (Table 1). All atopics had a positive history of allergic rhinitis, a positive skin prick test result for birch pollen and for the recombinant major birch pollen allergen Bet v 1(rBet v 1), as well as birch pollen–specific serum IgE (RAST classes ≥ 3; UniCAP®, Pharmacia & Upjohn, Freiburg, Germany). All volunteers were without medication for at least 15 days before blood sampling. The ethical committee of the Technical University of Munich approved the study, and volunteers were enrolled in the study after giving written informed consent. The investigation was conducted according to the Declaration of Helsinki. Controls were identified as subjects with a total IgE level of <25 kU/l and no specific serum IgE (RAST classes 0) to a panel of eleven allergens (*Dermatophagoides pteronyssinus*, cat, egg, milk, codfish, wheat, celery, *Phleum pratense* pollen, latex, birch pollen, mugwort).

Reagents and antibodies. rBet v 1 (*Betula verrucosa*, birch pollen allergen 1a) was purchased from Biomay (Vienna, Austria). Purity of approximately 99% was determined by SDS-PAGE and staining with Coomassie Brilliant Blue R-250. The lyophilized protein was reconstituted with ultrapure water to a concentration of 1 mg/ml and aliquoted. Aliquots were stored at −80°C to final use. Skin prick test solution was commercially available from Allergopharma (Reinbek, Germany).

Basophil activation and flow cytometry. For quantitative determination of rBet v 1–induced activation of human basophils, a commercially available test system was used (BASOTEST®, Orpegen Pharma, Heidelberg, Germany). Briefly, 100 µl heparinized whole blood was first stimulated with 20 µl phosphate buffered saline (PBS) buffer containing IL-3 for 10 min at 37°C and then incubated for 20 min with 100 µl of rBet v 1 alone or in combination with AERex at concentrations from 0.007 to 0.755 m^3 air equivalents. 1 µM of the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (f-MLP) was used as positive control, PBS solution served as negative background control. The degranulation process was stopped by storing the samples on ice for 5 min. 20 µl of a two-color antibody reagent consisting of PE-conjugated anti-IgE

TABLE 1
Characteristics of Atopic and Nonatopic Donors

Subjects	Gender	Age	SPT birch	RAST class birch/Bet v 1	Symptoms
Patients					
I	f	26	++++	4/3	yes
II	m	24	++++	3/3	yes
III	f	21	++++	4/4	yes
IV	f	39	++++	4/4	yes
V	m	38	++++	3/3	yes
VI	f	23	++++	3/3	yes
Controls					
I	f	24	neg.	0/0	no
II	m	32	neg.	0/0	no
III	f	26	neg.	0/0	no
IV	m	31	neg.	0/0	no
V	f	23	neg.	0/0	no

Note. Age, gender, specific IgE concentration (CAP-RAST class 0–6), and skin prick test (SPT) birch (++++: diameter of wheal > 6 mm, erythema > 20 mm) of individuals tested are shown; neg., negative.

and FITC-conjugated anti-gp53 were added and incubated for 20 min in an ice bath. After staining of basophils and removal of erythrocytes, the cells were washed twice and resuspended in 200 µl PBS. Flow cytometric analysis was performed within 2 h using a FACSCalibur flow cytometer (Becton Dickinson, Heidelberg, Germany) and CellQuest™ software. Basophilic granulocytes were gated by the presence of PE-conjugated anti-IgE, and expression of gp53 (CD63) was analyzed on this gated cell population. Acquisition was performed on 1000 cells for each sample, and results are given as the percentage of basophils expressing CD63. Results with more than 15% of activated basophils were regarded as positive, according to the manufacturer instructions (Orpegen Pharma, 1997). All basophil activation tests were performed outside the birch pollen season, which in Munich lasted from April 10 until May 1, 2003.

Statistical analysis. Data are expressed as the arithmetic means ± SD. A paired *t*-test was performed to compare differences of CD63 expression between AERex and controls. A p value of ≤ 0.05 was considered statistically significant.

RESULTS

Characterization of AERex

At least nine organic compound classes were identified by GC-MS analysis in AERex^{1d} and AERex^{5d} (Table 2). In particular, aromatic components such as PAHs and their derivates could be detected.

The mass concentration of the sum of all investigated PAHs in AERex^{1d} (1.14 ng/m^3) was roughly 20 times higher than in AERex^{5d} (0.06 ng/m^3). These values are similar to those values found in atmospheric particulate matter collected during previous studies in Munich (Lintelmann *et al*., 2005; Schauer *et al*., 2003; Schnelle-Kreis *et al*., 2001). Table 3 shows that, depending on the specific PAH, 10- to 1500-fold higher concentrations could be measured in AERex^{1d} than in AERex^{5d}. In particular, the benzo[*a*]pyrene (B[*a*]P) content in AERex^{1d} was found to be 165 ng/ml, whereas only marginal amounts of this compound and other EPA priority PAH pollutants were recovered in AERex^{5d}, which was collected during summer. No LPS reactivity was found in both filter extracts tested at a 1:100 dilution. AERex concentrations used in basophil activation assay were therefore below the detection limit of LAL test (0.05 E.U./ml).

TABLE 2
Qualitative Characterization of AERex^{1d} and AERex^{5d} by GC-MS Analysis

Compound classes
PAHs
PAH ketones and quinones
Nitro-PAHs
n-Alkanoic acids
n-Alkanes
Substituted phenols
Guaiacols and substituted guaiacols
Dicarboxylic acids
Aromatic carboxylic acids

Note. Solvent: DMSO.

TABLE 3
HPLC Analysis of Particle-Associated PAHs in AERex^{1d} and AERex^{5d}

PAHs	AERex^{1d} (ng/ml extract)[a]	AERex^{5d} (ng/ml extract)[b]
Acenaphthene*	n.d.	n.d.
Anthanthrene	17.00	0.22
Anthracene*	51.15	5.34
Benzo[*a*]anthracene*	256.38	2.30
Benzo[*b*]fluoranthene*	513.02	0.35
Benzo[*k*]fluoranthene*	201.69	0.26
Benzo[*g,h,i*]perylene*	76.05	0.69
Benzo[*a*]pyrene*	164.70	0.20
Benzo[*e*]pyrene	808.01	0.98
Chrysene*	546.32	3.88
Coronene	n.d.	0.26
Dibenzo[*a,h*]anthracene*	12.35	0.19
Fluoranthene*	945.04	96.35
Fluorene*	n.d.	4.40
Indeno[1,2,3-*c,d*]pyrene*	92.46	n.d.
Naphthalene*	n.d.	9.21
Phenanthrene*	391.89	62.58
Pyrene*	730.62	66.39
Total PAH content [ng/m^3]	1.14	0.06

Note. The samples were collected near (<10 m) a highly frequented six-lane road with 44,000 motor vehicles per day in the north of Munich, Germany. Solvent: DMSO; n.d., not detected.

[a] 4233.33 m^3 air/ml.

[b] 4152.73 m^3 air/ml.

*U.S. EPA priority pollutant.

CD63 Upregulation of Human Basophils Induced by rBet v 1

Antigen-induced regulation of CD63 expression on basophils of allergic donors was analyzed by incubation with serial dilutions of rBet v 1. Basophil activation was seen to be dose dependent in all patients, reaching a maximum upon stimulation with 4.5 ng/ml rBet v 1 (Fig. 1). For comparable studies on modulating effects of AERex, basic CD63 expression by rBet v 1 was adjusted individually for each donor to >10% and <50% basophils. As indicated by Figure 1, a dose range of 0.05–2.3 ng/ml rBet v 1 was found to be appropriate for basic activation in all atopic donors tested. None of basophil samples from healthy controls showed any upregulation of CD63 by rBet v 1.

Effect of AERex on Basophil Activation

Basophil CD63 expression was analyzed either after stimulation with rBet v 1 or upon AERex exposure, or both, to investigate possible synergy between AERex and IgE-mediated basophil activation in birch pollen–allergic donors and healthy controls.

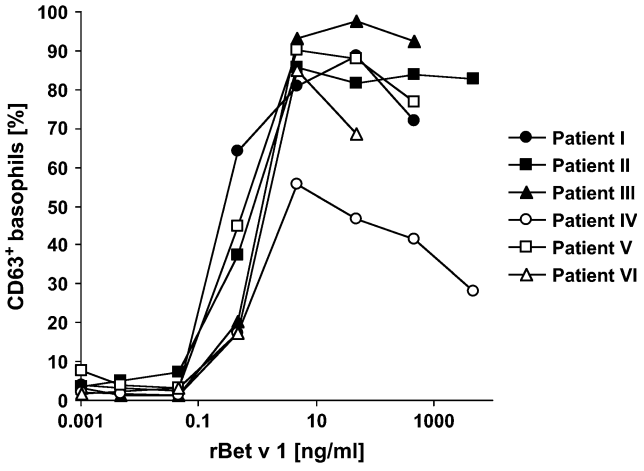

FIG. 1. Dose-response relationship of rBet v 1–induced CD63 upregulation in sensitized basophils. Each print represents the basophil activation of a single stimulation with allergen. A concentration of 4.5 ng/ml rBet v 1 induced a >50% upregulation in all patients. For all subsequent experiments concentrations ranging from 0.05 to 2.3 ng/ml rBet v 1 were used.

Activation with 0.05–2.3 ng/ml rBet v 1 induced 10% to 50% of basophils to upregulate CD63 (Fig. 2) except for donor I being below 10%. rBet v 1 upregulation of CD63 was not influenced by presence of DMSO (Fig. 3). After treatment with both AERex and allergen, basophil activation increased in all patients up to 90%, but was higher with AERex^{1d} than with stimulation with AERex^{5d} (Fig. 2). Compared to AERex^{5d}, 5- to 50-fold lower concentrations of AERex^{1d} were required for maximal effect on basophil activation, which is in accordance with the 20-fold higher total PAH content found in the 1-day sample (Table 3). AERex-induced enhancement of CD63 upregulation of rBet v 1 in sensitized basophils occurred in a dose-dependent manner as is shown exemplarily for one donor in Figure 4.

Both organic extracts synergized with rBet v 1 and significantly enhanced basophil activation in all birch pollen–allergic donors (Fig. 3). However, stimulation with AERex^{1d} or AERex^{5d} alone exhibited no effects on CD63 expression in basophils from atopic donors as well as from healthy controls, indicating no direct effect of AERex on human basophils in our study. In addition, both AERex extracts showed no enhancement of CD63 upregulation in f-MLP-stimulated basophils from birch pollen–allergic donors.

FACS analysis of propidium iodide–labeled cells revealed that both organic extracts were not toxic to basophils at dilutions of 1:1000 and higher (<0.1% DMSO). AERex-mediated enhancement of basophil activation was only seen at dilutions >1:5000.

DISCUSSION

Environmental particles (≤PM2.5: Particulate matter that is 2.5 μm and smaller in size) at ambient levels have been implicated in a great number of human health effects including atopic

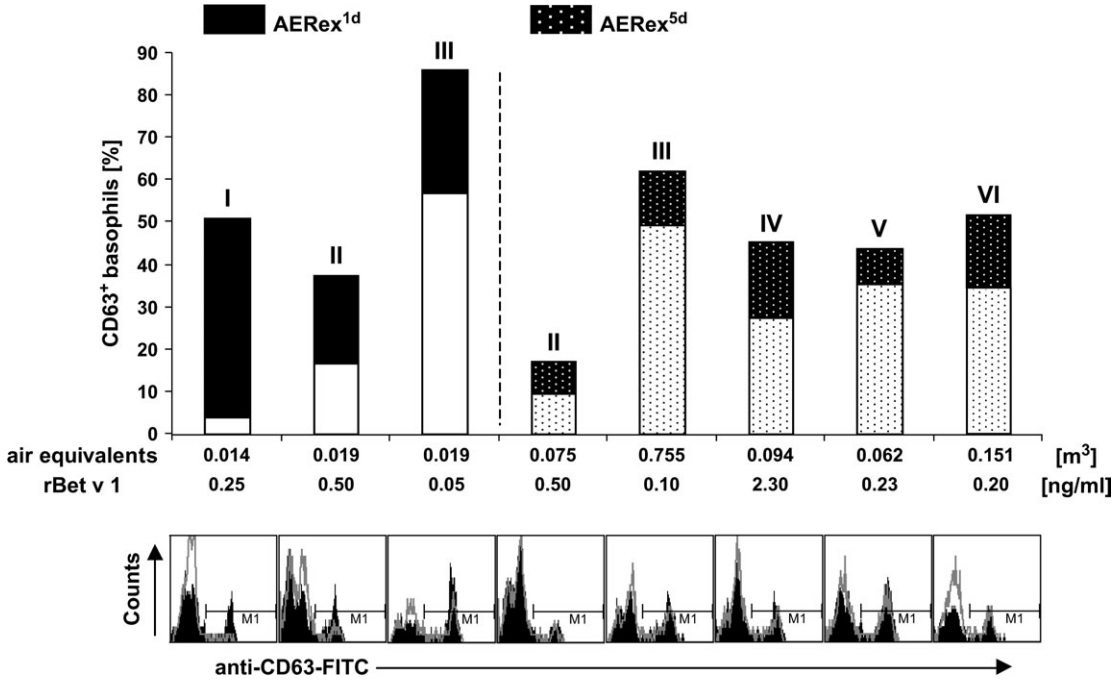

FIG. 2. Impact of AERex on rBet v 1–induced CD63 upregulation in human basophils from six birch pollen–allergic individuals. Patients II and III were tested with both AERex^{1d} and AERex^{5d}. Maximum effect is shown after treatment with rBet v 1 plus AERex (0.007–0.755 m^3, black columns or filled histograms) and rBet v 1 plus DMSO (<0.02%, white columns or unfilled histograms). Intra-assay variability of CD63 expression upon stimulation with rBet v 1 (0.05–2.30 ng/ml) was less than ±1.5%.

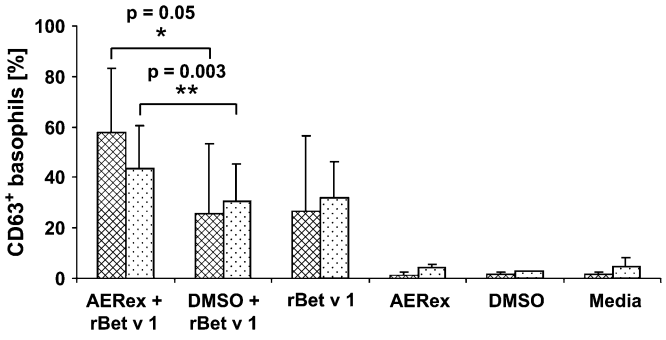

FIG. 3. AERex significantly enhances rBet v 1–induced basophil activation. Each column represents the mean ± SD of maximal dose-response relationships observed in three ($AERex^{1d}$) and five ($AERex^{5d}$) birch pollen–allergic individuals after treatment with AERex and rBet v 1.

diseases. An epidemiological association between allergic airway diseases and exposure to atmospheric pollutants has been demonstrated and suggested to be one factor in the increasing prevalence of asthma and rhinoconjunctivitis (Heinrich and Wichmann, 2004; Krämer *et al*., 2000; Studnicka *et al*., 1997; van Vliet *et al*., 1997). In human subjects, studies clearly demonstrated that diesel exhaust particles (DEPs) participate in the development of allergic airway inflammation by enhancing specific IgE and Th2 cytokine production in response to common allergens (Diaz-Sanchez, 1997; Diaz-Sanchez *et al*., 1996, 1997). However, there is growing experimental evidence that organic substances adsorbed to the particles (polycyclic aromatic hydrocarbons, PAHs) are responsible for adjuvant effects of DEPs independently from the particle nature (Bömmel *et al*., 2000, 2003; Devouassoux *et al*., 2002; Hitzfeld *et al*., 1992; Suzuki *et al*., 1993; Takenaka *et al*., 1995; Tsien *et al*., 1997). Due to their lipophilicity, such compounds are able to interact with a wide range of cells involved in the immune response, including basophilic leukocytes (Kepley *et al*., 2003).

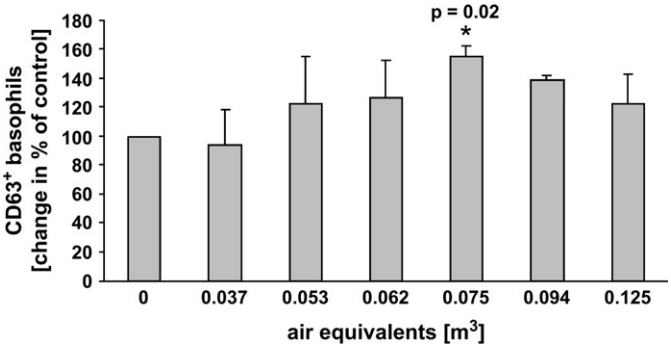

FIG. 4. Dose-dependent enhancement of rBet v 1–induced basophil activation by $AERex^{5d}$. Basophils of donor II were incubated with rBet v 1 and different concentrations of $AERex^{5d}$ (0.755–0.007 m^3) to exemplify the dose-response relationship of AERex on rBet v 1–induced basophil activation. The mean ± SD of three independent experiments is shown, and results are expressed as the percentage of maximal CD63 expression (100%) upon stimulation with 0.3 ng/ml rBet v 1.

In the present study we investigated whether AERex directly affects human basophils or synergizes with rBet v 1 in stimulating basophil activation. For these *in vitro* experiments, we prepared and used two organic extracts of urban particulate matter (≤PM2.5) comprising quantitatively different PAH patterns.

Several studies have provided new insights into the possible involvement of basophils in initiation and amplification of the specific IgE-dependent allergic inflammatory response and fueled speculations that extend beyond their recognized role as effector cells (Devouassoux *et al*., 1999; Falcone *et al*., 2000). During the course of allergic reactions, basophils have been shown to migrate into tissues participating in inflammatory reactions (Durham, 1998). Upon crosslinking of high affinity IgE receptors (FcεRI) or in response to other stimuli, basophils rapidly release mediators such as histamine and leukotrienes (Marone, 1988) as well as regulatory cytokines such as IL-4 (Arock *et al*., 1993; Brunner *et al*., 1993) and IL-13 (Gibbs *et al*., 1996; Li *et al*., 1996; Ochensberger *et al*., 1996). Organic extracts of DEPs have been shown to induce IL-4 expression and histamine release in highly enriched basophil populations, suggesting basophils to be a direct target for organic compounds present in diesel soot (Devouassoux *et al*., 2002).

We tested basophils from nonatopic as well as from allergic donors for synergy between AERex and allergen, because basophils from each donor group have differing activation requirements (Devouassoux *et al*., 1999). Our data demonstrate that both AERex extracts tested significantly enhance rBet v 1–induced basophil activation in all birch pollen–allergic subjects at ambient concentrations. This effect of AERex occurs in a dose-dependent manner. Synergy was only observed in basophils from atopic individuals, but not in healthy controls. This is in good accordance with studies reporting synergy of DEPs and antigen on Th2-type cytokine production after *in vivo* nasal or bronchial challenges in allergic subjects (Diaz-Sanchez *et al*., 1996, 1997). A similar effect of organic extract of diesel exhaust particles (DEPex) on allergen-induced degranulation of mast cells has been previously described (Diaz-Sanchez *et al*., 2000). Recent findings showed that DEPex is able to efficiently initiate IgE-independent basophil cytokine production and histamine release (Devouassoux *et al*., 2002). In contrast to this study, we used organic extracts of freshly sampled urban particulate matter (≤PM2.5) with different PAH content. Separation of urban PM10 into fine and coarse size particles revealed that the main proinflammatory response (TNF, IL-6, COX-2) in alveolar macrophages was driven by material present in the coarse PM, containing 90 to 95% of the stimulatory material in PM10 (Becker *et al*., 2005). Primary epithelial cells also responded to the coarse fraction with higher levels of IL-8 and COX-2 expression than that induced by fine or ultrafine PM. All size PM induced oxidant stress in epithelial cells. Compared to ambient PM, diesel PM induced only a minimal cytokine response in both alveolar macrophages and epithelial cells. Both coarse and

fine ambient air PM were also found to inhibit LPS-induced TNF release, while silica, volcanic ash, or carbon black had no inhibitory effect. Diesel particles did not affect cytokine mRNA induction nor protein accumulation but interfered with the release of cytokine from the cells. Ambient coarse and fine PM, on the other hand, inhibited both mRNA induction and protein production. Furthermore, Lin and coworkers (2005) found that nanoparticles contain more of traffic-related metals (Pb, Cd, Cu, Zn, Ba, and Ni) than particles of other sizes, although crustal metals accounted for over 90% of all the particulate metals. The Ag, Ba, Cd, Pb, Sb, V, and Zn contents in nanoparticles were strongly associated with diesel fuel. Divergent chemical composition and different distribution of fine and ultrafine particles in ambient PM and diesel PM might explain the absence of synergistic effects of DEPex and allergen on basophils in Devouassoux's study (Devouassoux *et al.*, 2002).

Strongest basophil activation was monitored upon stimulation with AERex^{1d}, collected in winter 2003, which comprises a 20-fold higher total PAH content than AERex^{5d}, collected in summer 2003. This suggests that the biological activity of AERex is markedly correlated to the content of certain organic components in urban aerosols, in particular PAHs. PAH concentrations fluctuate significantly over the year, with generally higher PAH levels in the winter months (Caricchia *et al*., 1999; Schauer *et al*., 2003). At low temperatures motor vehicles need a longer time to reach the optimum working temperature, which is characterized by an increased emission of PAHs (Lintelmann *et al*., 2005). In addition, significant changes of PAH levels at the end and beginning of the heating period indicate an influence of PAHs emitted by domestic heating. Inversion layers, which are typical for winters in the Munich area, inhibit exchange in the atmosphere, leading to accumulation of pollutants in the lowest layer (Lintelmann *et al*., 2005; Schauer *et al*., 2003). Furthermore, spring and summer in 2003 were extremely hot, leading to high UV radiation and high ozone concentrations. This caused photodegradation and oxidation of PAHs and easier atmospheric dispersion of pollutants (Lintelmann *et al*., 2005).

Overall, the composition of the organic phase of urban aerosols is dependent on seasonality of sampling, location, weather conditions, and other factors. This phase consists of over 20,000 different compounds, of which only one third could be identified to date. In our extracts, we can not exclude that other compounds than PAHs contribute to the observed effects. However, HPLC analysis of AERex and comparison with other studies investigating adjuvant effects of polyaromatic compounds (Bömmel *et al*., 2000; Kepley *et al*., 2003; Tsien *et al*., 1997) strongly indicate that, for the most part, PAHs contribute to the enhancement of rBet v 1–induced basophil activation in birch pollen–allergic patients. Since both organic extracts were sampled in different seasons and over different periods of time, this enhancement has to be considered a general effect of AERex on human basophils.

The bioavailability of PM-associated PAHs is known from cancer studies. To explore the basic relationship between a model PAH and a typical carrier particle, Gerde and coworkers (2001) investigated the rate and extent of release and metabolic fate of B[*a*]P adsorbed on the carbonaceous core of diesel soot. The native organic content of the soot had been denuded by toluene extraction. Exogenous B[*a*]P was adsorbed onto the denuded soot as a surface coating corresponding to 25% of a monomolecular layer. Dogs were exposed by inhalation to an aerosol bolus of the soot-adsorbed B[*a*]P. Following deposition in the alveolar region, a fraction of B[*a*]P was rapidly desorbed from the soot and quickly absorbed into the circulation. Release rates then decreased drastically. When coatings reached approximately 16% of a monolayer, the remaining B[*a*]P was not bioavailable and was retained on the particles after 5.6 months in the lung. However, the bioavailability of particles transported to the lymph nodes was markedly higher; after 5.6 months the surface coating of B[*a*]P was reduced to 10%. B[*a*]P that remained adsorbed on the soot surface after this period was approximately 30% parent compound. In contrast, the rapidly released pulse of B[*a*]P, which was quickly absorbed through the alveolar epithelium after inhalation, appeared mostly unmetabolized in the circulation, along with low concentrations of phase I and phase II B[*a*]P metabolites. The results indicate that absorption through the alveolar epithelium is an important route of entry to the circulation of organic compounds, in particular PAHs.

PAHs are bifunctional inducers that lead to the production of reactive oxygen species (ROS) through the induction of cytochromes P4501A (Prochaska and Talalay, 1988) and monofunctional inducers (quinones and derivatives) that can directly drive oxidative stress (Pinkus *et al*., 1996). ROS can activate a number of signaling pathways and cellular events. These effects are suspected to cause or to aggravate health impairments like chronic inflammatory processes or acute symptomatic responses in the respiratory tract (Dellinger *et al*., 2001; Li *et al*., 2002; Saxon and Diaz-Sanchez, 2005; Squadrito *et al*., 2001). The involvement of such metabolic pathways in human polymorphonuclear granulocytes has been previously reported (Devouassoux *et al*., 2002; Hitzfeld *et al*., 1997) and could also account for the modulating effects of AERex on human basophils (Kepley *et al*., 2003). Recent studies have shown that genetic variants of glutathione-S-transferases, which participate in ROS metabolism and detoxification of xenobiotics, affect susceptibility to DEP's enhancement of allergic responses (Gilliland *et al*., 2004). This may also reflect on the cellular level of basophils we studied.

In summary, the capability of AERex to increase Bet v 1–induced basophil activation in birch pollen–allergic subjects at ambient concentrations suggests an important role of organic compounds, predominantly PAHs, in the maintenance and aggravation of IgE-mediated allergic diseases. Since basophils represent a major source of early IL-4 production in allergic

patients after specific antigen activation (Devouassoux *et al*., 1999), synergy between AERex and natural Bet v 1 exposure may also contribute to primary sensitization to other ubiquitous aeroallergens.

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