

This article was downloaded by: [Advanced Institute of Sanita]

On: 1 June 2010

Access details: Access Details: [subscription number 909943353]

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Toxicology and Environmental Health, Part A

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713667303>

Fine Ambient Particles from Various Sites in Europe Exerted a Greater IgE Adjuvant Effect than Coarse Ambient Particles in a Mouse Model

Torunn Alberg^a; Flemming R. Cassee^b; Else-Carin Groeng^a; Erik Dybing^a; Martinus Løvik^a

^a Norwegian Institute of Public Health, Division of Environmental Medicine, Oslo, Norway ^b National Institute for Public Health and the Environment, Centre for Environmental Health Research, Bilthoven, The Netherlands

To cite this Article Alberg, Torunn , Cassee, Flemming R. , Groeng, Else-Carin , Dybing, Erik and Løvik, Martinus(2009) 'Fine Ambient Particles from Various Sites in Europe Exerted a Greater IgE Adjuvant Effect than Coarse Ambient Particles in a Mouse Model', Journal of Toxicology and Environmental Health, Part A, 72: 1, 1 – 13

To link to this Article: DOI: 10.1080/15287390802414471

URL: <http://dx.doi.org/10.1080/15287390802414471>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Fine Ambient Particles from Various Sites in Europe Exerted a Greater IgE Adjuvant Effect than Coarse Ambient Particles in a Mouse Model

Torunn Alberg¹, Flemming R. Cassee², Else-Carin Groeng¹, Erik Dybing¹, and Martinus Løvik¹

¹Norwegian Institute of Public Health, Division of Environmental Medicine, Oslo, Norway, and ²National Institute for Public Health and the Environment, Centre for Environmental Health Research, Bilthoven, The Netherlands

In the European Union (EU)-funded project Respiratory Allergy and Inflammation due to Ambient Particles (RAIAP), coarse and fine ambient particulate matter (PM) was collected at traffic dominated locations in Oslo, Rome, Lodz, and Amsterdam, in the spring, summer, and winter 2001/2002. PM was also collected in de Zilk, a rural seaside background location in the Netherlands. The aim of this study was to screen the ambient PM fractions for allergy adjuvant activity measured as the production of allergen- (ovalbumin-) specific immunoglobulin (Ig) E following subcutaneous (sc) injection into the footpad of mice. A second aim was to determine whether the 6-d popliteal lymph node (PLN) assay can be used to detect an allergy adjuvant activity. Allergy screening for IgE adjuvant activity showed that in the presence of ovalbumin (Ova) 12 out of 13 of the fine ambient PM fractions exerted a significant IgE adjuvant activity. In contrast, only 3 out of 13 of the coarse PM fractions had significant adjuvant activity. Overall, fine ambient PM exerted significantly greater IgE adjuvant activity per unit mass than coarse PM. No significant differences were observed between locations or seasons. Substantial higher levels of specific components of PM such as vanadium (V), nickel (Ni), zinc (Zn), ammonium (NH₄), and sulfate (SO₄) were present in the fine compared to coarse PM fractions. However, differences in the content of these components among fine PM fractions did not reflect the variation in the levels of IgE anti-Ova. Still, when comparing all seasons overall, positive correlations were observed between V, Ni, and SO₄ and the allergen specific IgE levels. The

PLN responses (weight and cell number) to Ova and ambient PM in combination were significantly higher than to Ova or PM alone. Still, the PLN assay appears not to be useful as a quantitative assay for screening of allergy adjuvant activity since no correlation was observed between PLN responses and allergen specific IgE levels. In conclusion, fine ambient PM fractions consistently were found to increase the allergen-specific IgE responses more than the coarse ones. Our finding is in agreement with the notion that traffic-related air pollution contributes to the disease burden in asthma and allergy, and points to fine and ultrafine ambient PM as the most important fractions in relation to allergic diseases.

Air pollution has been suggested as a causative or promoting factor of respiratory allergies (D'Amato et al., 2005; Gilmour et al., 2006). The RAIAP project has focused on one specific type of air pollutant, namely, ambient particulate matter (PM). Although the exposure to ambient PM is substantial in urban environments throughout Europe, the prevalence of respiratory allergies has been reported to differ, with a higher prevalence in the western than eastern countries (Braback et al., 1994; ISAAC & the International Study of Asthma and Allergies in Childhood Steering Committee, 1998; Janson et al., 2001; von Mutius et al., 1994). After the reunification of East and West Germany, however, an increase in hay fever and atopic sensitization occurred in the former Eastern Germany (von Mutius et al., 1998; Weiland et al., 1999). Since the 1950s air pollution has differed between European countries, with the eastern type of pollution being dominated by particles and sulfur dioxide from coal combustion and the western type by smaller particles and oxides of nitrogen largely derived from combustion of fossil fuels. During recent years, however, there has been a change in the air pollution in Europe: The burning of coal in the east being reduced, and diesel emissions in these locations increasing (Ebel et al., 2001; Kreyling et al., 2003; Weiland et al., 1999). Traffic-related air pollution has been speculated to be one

Received 15 April 2008; accepted 8 August 2008.

We thank John Boere and Paul Fokkens, National Institute for Public Health and the Environment, the Netherlands, who were responsible for organization of the RAIAP sampling campaign. At the Norwegian Institute of Public Health, Åse Eikeset and Berit A. Stensby are acknowledged for excellent technical assistance. Sincere thanks also go to Unni Cecilie Nygaard for critical reading of the manuscript and for valuable statistical discussions. RAIAP was a European Commission 5th Framework program shared-cost research project, QLK-CT-2000-00792.

Address correspondence to Torunn Alberg, Norwegian Institute of Public Health, Division of Environmental Medicine, PO Box 4404 Nydalen, N-0403, Oslo, Norway. E-mail: Torunn.Alberg@fhi.no

reason for the rise in allergic diseases observed in Europe during the past decades, and it was recently shown that exposure to traffic-related air pollutants exerts adverse effects in relation to atopic diseases as well as allergic sensitization (Morgenstern et al., 2008). In addition to locality differences, seasonal variability in air pollutants was observed, with high PM₁₀ concentrations during the winter compared to summer months, due to residential heating, inversions, and less complete combustion by automobile engines during the colder months of the year (Aalto et al., 2005; Ebelt et al., 2001; Harrison and Jones, 2005; Namork et al., 2004; Paoletti et al., 2002).

A main objective of the RAIAP project was to address whether qualitative differences in particulate air pollution at different locations in part might explain differences in prevalence or severity of respiratory allergies throughout Europe. For this purpose, coarse (2.5–10 µm in aerodynamic diameter) and fine (0.1–2.5 µm in aerodynamic diameter) ambient PM was collected nearby busy streets in four European cities: a northern one—Oslo, a southern one—Rome, an eastern one—Lodz, and a western one—Amsterdam. PM was collected in three seasons: spring, summer, and winter in the years 2001/2002. Moreover, in the fall 2001, PM was collected in de Zilk, a rural seaside background location in the Netherlands with no major traffic road in the vicinity. In the present study the PM fractions were screened in a mouse model for their allergy adjuvant activity, measured as the allergen-specific immunoglobulin (Ig) E response following subcutaneous sc injection into the footpad of BALB/cA mice. Diesel exhaust particles (DEP) were included as a positive adjuvant control. A second aim was to determine whether the 6-d PLN assay might be used for allergy screening since (1) it is highly sensitive (i.e. producing a measurable immune response), (2) it is simple to perform because the immune response is focused to one single well-defined and easily detectable lymph node, and (3) results are reproducible. For this purpose the results obtained in the PLN assay were compared with the production of allergen specific IgE after sc injection into the footpad of mice. A short account for this study has appeared previously (Dybing et al., 2004).

METHODS

Animals

Inbred 6-wk-old female BALB/cA mice, weighing approximately 15 g each, were obtained from Taconic M&B A/S (Ry, Denmark). Mice were distributed in groups of 8 animals per cage (type III macrolon) containing Beekay GLP bedding (B&K Universal A/S, Nittedal, Norway), and rested for 1 wk before entering the experiments. The cages were placed in filter cabinets (Scantainers) and mice exposed to a 12/12-h light/dark cycle, a temperature of $21 \pm 2^\circ\text{C}$, and a relative humidity of 40–60%. The mice were given pelleted food (RM1, SDS, Essex, UK) and tap water ad libitum. The experiments were performed in conformity with the laws and regulations

controlling experiments with live animals in Norway, and were approved by the local officer of the Experimental Animal Board under the Ministry of Agriculture in Norway.

Particulate Material

Ambient PM was collected in Oslo, Rome, Lodz, and Amsterdam during the spring, summer, and winter in the years 2001/2002. In addition, PM was collected in de Zilk, a seaside background location in the Netherlands, in the fall 2001. PM was collected as described by Cassee et al., (2003). In short, two high-volume cascade samplers with a multistage slit nozzle impactor were used to collect coarse and fine PM fractions into polyurethane foams. PM was extracted with methanol from the polyurethane foams (Demokritou et al., 2002; Sillanpaa et al., 2003). Standard Reference Material 1650 (DEP) was obtained from the National Institute of Standards and Technology (Gaithersburg, MD), whereas Ottawa dust (EHC-93) was kindly provided by Dr. Renauld Vincent (Environmental Health Directorate, Health Canada, Ottawa, Ontario, Canada). PM was suspended in Hanks balanced salt solution (HBSS) and stirred at 18°C overnight using a magnetic stirrer. When appropriate, allergen was added to the suspension simultaneously with particles.

Particle Characterization

Characterization of the collected ambient PM fractions was performed at the National Institute of Public Health and the Environment, the Netherlands, and is described in detail by Cassee et al. (2003). In summary, elemental composition of the PM was analyzed with inductively coupled plasma mass spectrometry. Secondary aerosols were detected with ion chromatography (Cl, NO₃, and SO₄) or photometry (NH₄), and anions were analyzed using a Dionex guard column (AG-4A), separation column (Dionex AS-4A), and pulsed electrochemical detector (Dionex-PED). Polycyclic aromatic hydrocarbons (PAH) were analyzed on a 30-m, 0.25-mm, WCOT DB-5MS column in a Fisons 8000 series gas chromatograph equipped with an Inter-science MD800 mass spectrometer with electron ionization (EI) in SIR mode.

Experimental Design

An overview of the experimental design is shown in Figure 1. All experiments were performed with groups of 8 mice injected into the right hind footpad in a heel-to-toe direction using a 100-µl Hamilton syringe (Hamilton Bonaduz AG, Switzerland) with a 30-G needle. Suspensions (20 µl) of ambient particles in HBSS (200 and 100 µg single dose per animal in the antibody and PLN experiments, respectively) were given together with the model allergen ovalbumin (Ova) (grade VII, Sigma, St. Louis, MO, 50 µg single dose per animal). DEP was included as a positive control (50 µg single dose per animal, 30 µg in the spring antibody experiment). In the PLN assay,

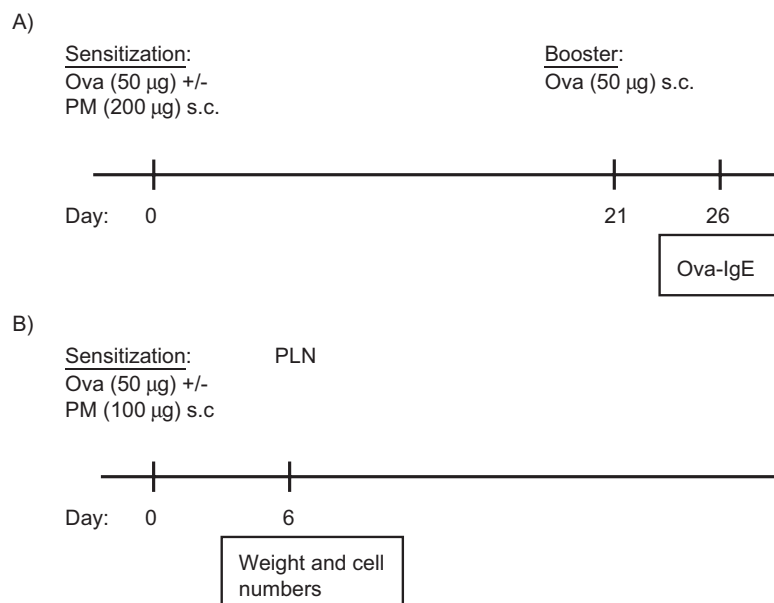


FIG. 1. Experimental design for examination of effects of ambient PM on (A) allergen-specific IgE production and (B) PLN weight and cell number responses after sc injection of Balb/cA mice into the hind footpad. See Methods section for details.

ambient particles were also given in the absence of Ova. In the antibody experiments untreated and HBSS-treated mice were included as controls and ambient dust collected in Ottawa (EHC-93) was administered as a generally available reference particle. All particle fractions collected in the same season were examined in one antibody production experiment, whereas the coarse and fine PM fractions for practical reasons were examined in separate experiments in the PLN assay.

The PLN Assay

Six days after sensitization mice were killed by cervical dislocation under CO₂ anesthesia, and PLNs from both hind legs were dissected out and kept on ice in HBSS. Further preparation of the lymph nodes was carried out as described by Løvik and coworkers (1997). In short, after removal of excess fat and connective tissue, the lymph nodes were weighed. Each lymph node was then teased into a single-cell suspension by means of two 18-G needles, and the number of cells was determined in a Beckman Coulter Counter (Beckman Coulter, Inc., Fullerton, CA). Weight and cellular indices were calculated by dividing the value of the right (inoculated) lymph node with that of the left (noninoculated).

Allergen-Specific IgE Assay

For measurements of allergen-specific IgE antibodies, mice were given a second injection of Ova 20 d after the first one, and 5 d later the mice were anesthetized with CO₂ and blood drawn from the heart. Sera were prepared and stored at -20°C until analyzed by enzyme-linked immunosorbent assay

(ELISA). The ELISA for Ova-specific IgE has been described previously (Lovik et al., 1997; Ormstad et al., 1998). In short, microtiter plates coated with rat anti-mouse IgE (LO-ME-3, Experimental Immunology Unit, University of Louvain, Belgium) were allowed to capture IgE. Capture of IgE antibodies was followed by incubation with biotinylated Ova and thereafter with preformed complexes of streptavidin and biotinylated alkaline phosphatase, and finally with *p*-nitrophenyl phosphate. Optical density was measured at 405 nm and the antibody concentration calculated in arbitrary units per milliliter (AU/ml).

A standard curve was made on each IgE plate using a serum pool from BALB/cA mice immunized with Ova and Al(OH)₃. Ova and Al(OH)₃ were given intraperitoneally on d 0, 20, 34, and 41 as single doses of 10 µg and 2 mg, respectively. Mice were anesthetized with CO₂ and blood drawn from the heart on d 48. After preparation sera were stored at -20°C.

Statistical Analysis

Statistical analysis was performed using SigmaStat for Windows Version 2.03 (Statistical Solutions, Saugus, MA). Nonparametric analyses on ranks were performed on the IgE antibody data sets, which were nonnormally distributed. Moreover, some antibody values were outside the detection range of the ELISA. Variance was introduced into the groups in which nearly all values were below the detection level of the ELISA, by creating normally distributed random numbers with standard deviation below 0.02. Parametric analyses were performed on the PLN data sets that were normally distributed. Each separate experiment was analyzed using one-way analysis of variance,

and adjuvant activity was examined by multiple comparisons versus the Ova group (Dunn's and Dunnett's method for the nonparametric and parametric responses, respectively). To determine whether the PLN response in the presence of Ova was significantly greater than in the absence of Ova, and for which particle sampling locations, two-way analysis of variance was performed (Holm-Sidak method). To find out whether the popliteal response to the combination of Ova and ambient PM was due to a synergistic or additive effect of Ova and particles, the mean index values of all groups following subtraction of one (reflects no stimulation) were calculated. The mean index value of the Ova group was then added to the mean index value of the individual particle groups and the sum obtained compared by a Student's *t*-test to the mean index values of the particle and Ova groups. The importance of sampling site, season, and particle size for the PLN and IgE antibody responses was determined following a three-way analysis of variance (Holm-Sidak method). To determine the association between the allergen-specific IgE levels and PLN responses (weight and cell number), as well as between allergen-specific IgE levels and specific components of PM, Spearman's rank order correlation analysis was performed. Correlation coefficients below .6 were not considered to be relevant. The limit for statistical significance was set at $p \leq .05$.

RESULTS

Allergen-Specific IgE Responses

To examine the allergy adjuvant activity of the ambient PM, its ability to increase an allergen specific IgE response was analyzed. The results obtained are presented in Figure 2. Upper, middle, and lower panels show the results for ambient PM collected in the spring, summer, and winter season, respectively. The result obtained for the PM collected at the rural seaside background location de Zilk is shown in the lower panel. A weak IgE antibody response to Ova without PM was observed. However, in the presence of Ova 12 out of 13 of the fine ambient PM fractions significantly increased the IgE anti-Ova response in the dose examined (200 μg single dose per animal). In contrast, only 3 out of 13 of the coarse ambient PM fractions had significant IgE adjuvant activity. No adjuvant activity was observed with a lower dose of ambient PM (50 μg single dose per animal, data not shown). The Ottawa dust gave a rather weak IgE antibody response compared with the European ambient PM. The dose of the positive control particles (DEP) was increased from 30 to 50 μg per mouse in the summer and winter experiments, after it was found that the dose of 30 μg did not significantly enhance IgE anti-Ova responses compared to Ova alone (upper panel). It was noticeable that both the coarse and fine PM fractions from de Zilk did not markedly differ from the other locations.

Three-way analysis of variance showed that the fine PM fractions in combination with Ova produced a significantly

greater allergen specific IgE response than the coarse ones. However, no significant differences in the IgE antibody response were observed between locations or seasons.

PLN Responses

Figure 3 shows the popliteal lymph node weight responses to coarse (white columns) and fine (gray columns) ambient PM, collected in Oslo, Rome, Lodz, and Amsterdam, in the spring (upper panel), summer (middle panel), and winter (lower panel), respectively. The results obtained for the PM collected at the background location de Zilk are shown in the lower panel. It should be noticed that the coarse and fine ambient PM for practical reasons were examined in separate experiments.

Only weak lymph node weight responses to Ova without PM were obtained. The same was found for the majority of PM fractions in the absence of Ova. With the exception of several coarse summer PM fractions and the fine winter PM fraction from Oslo, the lymph node weight responses to most PM fractions were significantly increased in the presence of Ova. The response turned out to be synergistic, not just an additive effect of Ova and particles. Similar results were obtained for the popliteal lymph node cellular responses (data not shown), with the correlation coefficient between weight and cellular PLN responses being .8. There were, however, no significant correlations between the IgE anti-OVA and PLN responses, with the correlation coefficient being .2 for both the weight and cellular lymph node responses.

Particle size was an important determinant for popliteal weight responses for the PM fractions collected in the summer. No other statistically significant differences were obtained in the PLN responses (weight and cell number) for PM in combination with Ova according to variance analysis of PM sampling location, season, and size.

Allergen-Specific IgE Responses in Relation to Chemical Components of PM

An extensive chemical characterization of the ambient PM fractions collected in the RAIAP project showed that the PM fractions differed among the sampling sites with respect to both mass concentration and composition (Cassee et al., 2003). Higher concentrations ($\mu\text{g}/\text{m}^3$) of ambient PM were found in Lodz and Rome compared to Oslo, Amsterdam, and de Zilk. There was a clear predominance of combustion particles in Lodz, particularly in the winter samples. The relationship between aluminum (Al) and the transition metals iron (Fe), copper (Cu), zinc (Zn), vanadium (V), and nickel (Ni) in the PM and the production of allergen-specific IgE is shown in Figure 4. In general, the highest concentrations of metals were found in Rome (Cassee et al., 2003), with the exception of Zn, which was highest in Lodz, particularly in the winter. Al, Fe, and Cu are typical for crustal material and were usually higher

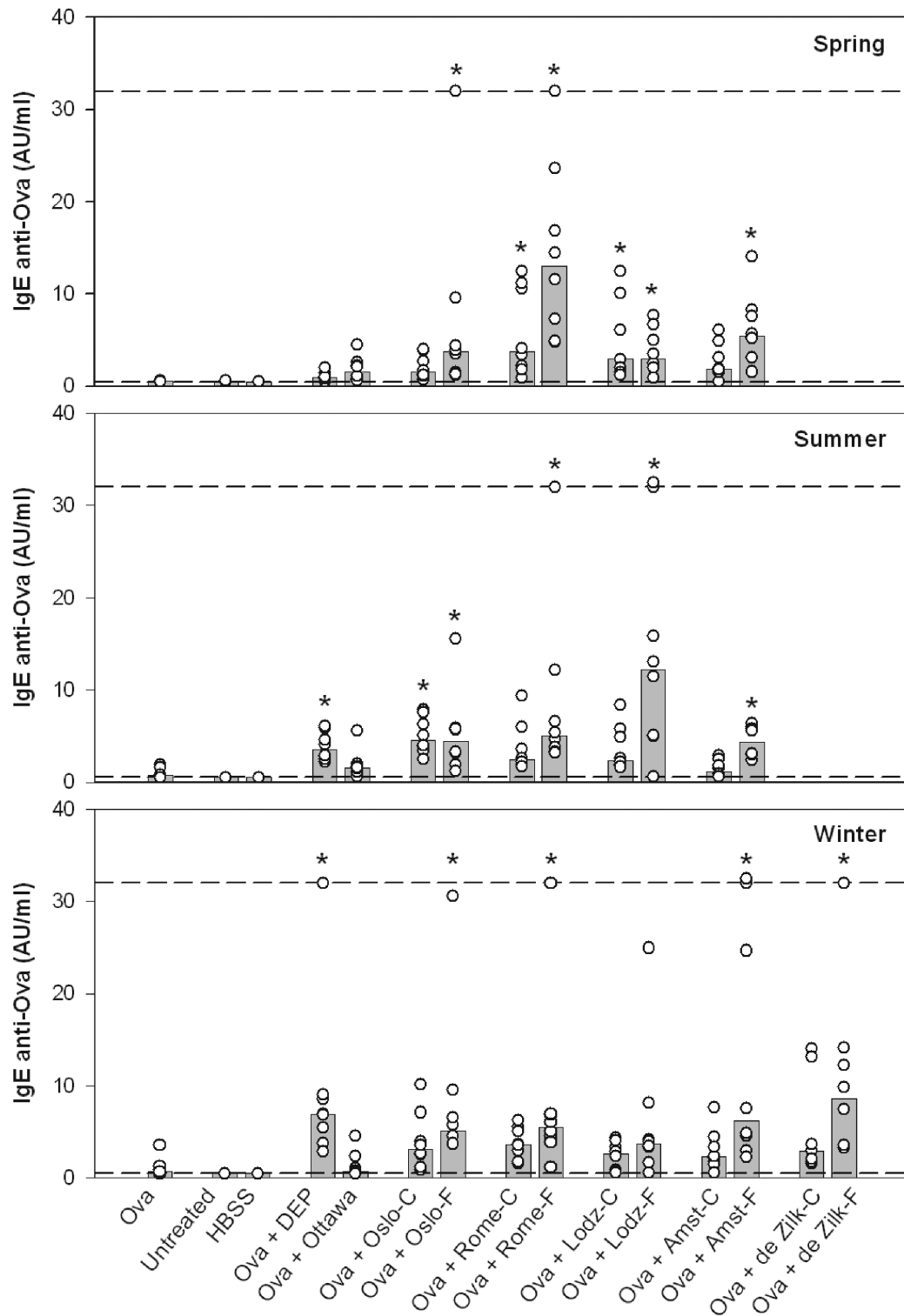


FIG. 2. Levels of IgE anti-Ova in BALB/cA mice 5 days after footpad inoculation with Ova, 25 days after inoculation of Ova and PM into the same footpad. The upper, middle, and lower panels show the results obtained for ambient PM collected in the spring, summer, and winter season, respectively. Individual results (circles) and medians (columns) for groups of eight mice are shown. The dotted lines indicate the lower and upper detection limits of the ELISA. The stars indicate a statistically significant difference compared to Ova ($p \leq .05$).

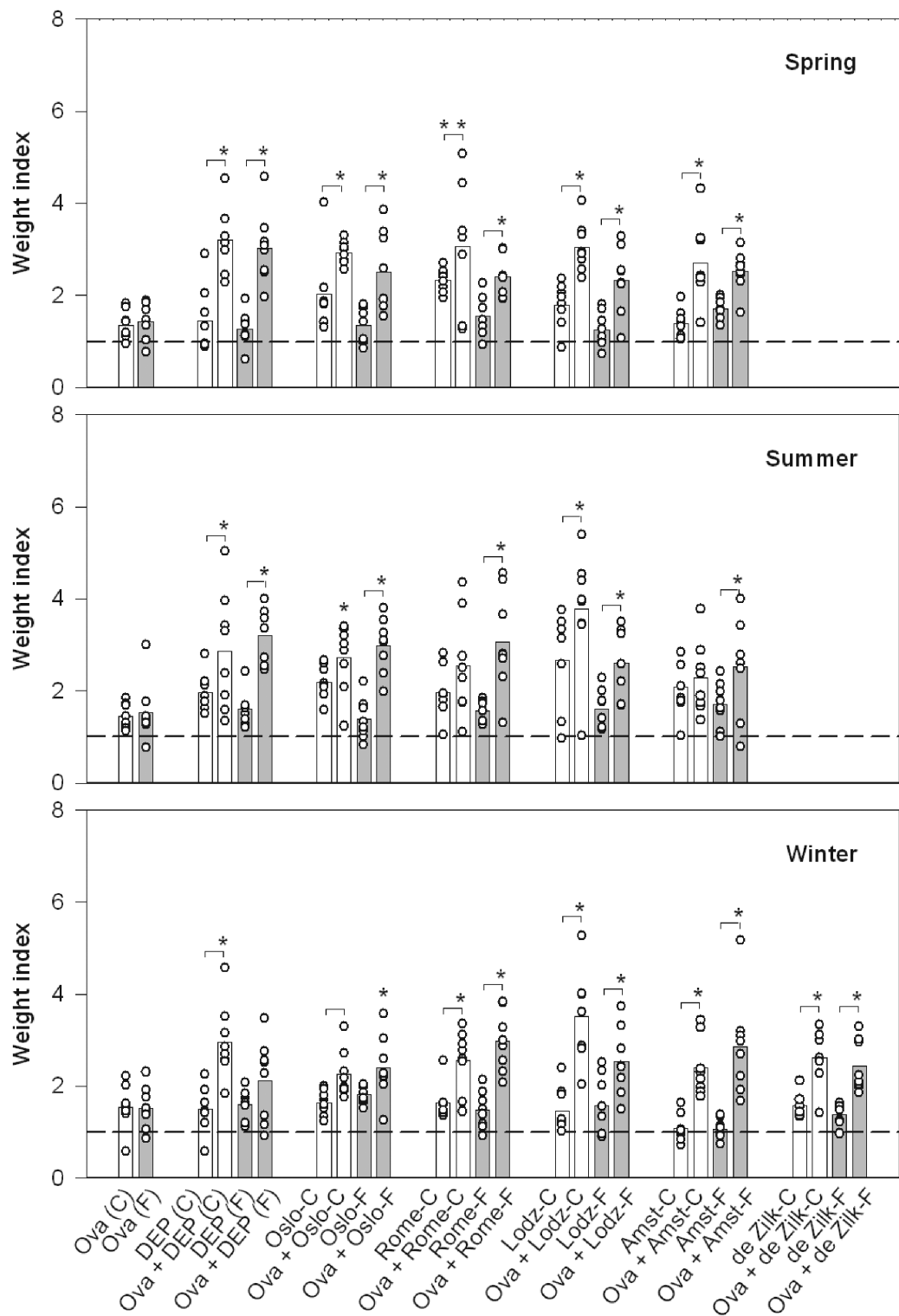


FIG. 3. Weight indices in the PLN 6 d after footpad inoculation of BALB/c mice with Ova and ambient PM fractions, separately or in combination. The upper, middle, and lower panels show the results obtained for ambient PM collected in the spring, summer, and winter season, respectively. Individual results (circles) and means (columns) for groups of eight mice are shown. Coarse PM (white bars) and fine PM (gray bars) were examined in separate experiments. The dotted line indicates 1, which signifies no reaction. The stars indicate a statistically significant difference compared to the respective Ova group, whereas brackets denote a significant difference between the groups indicated ($p \leq .05$).

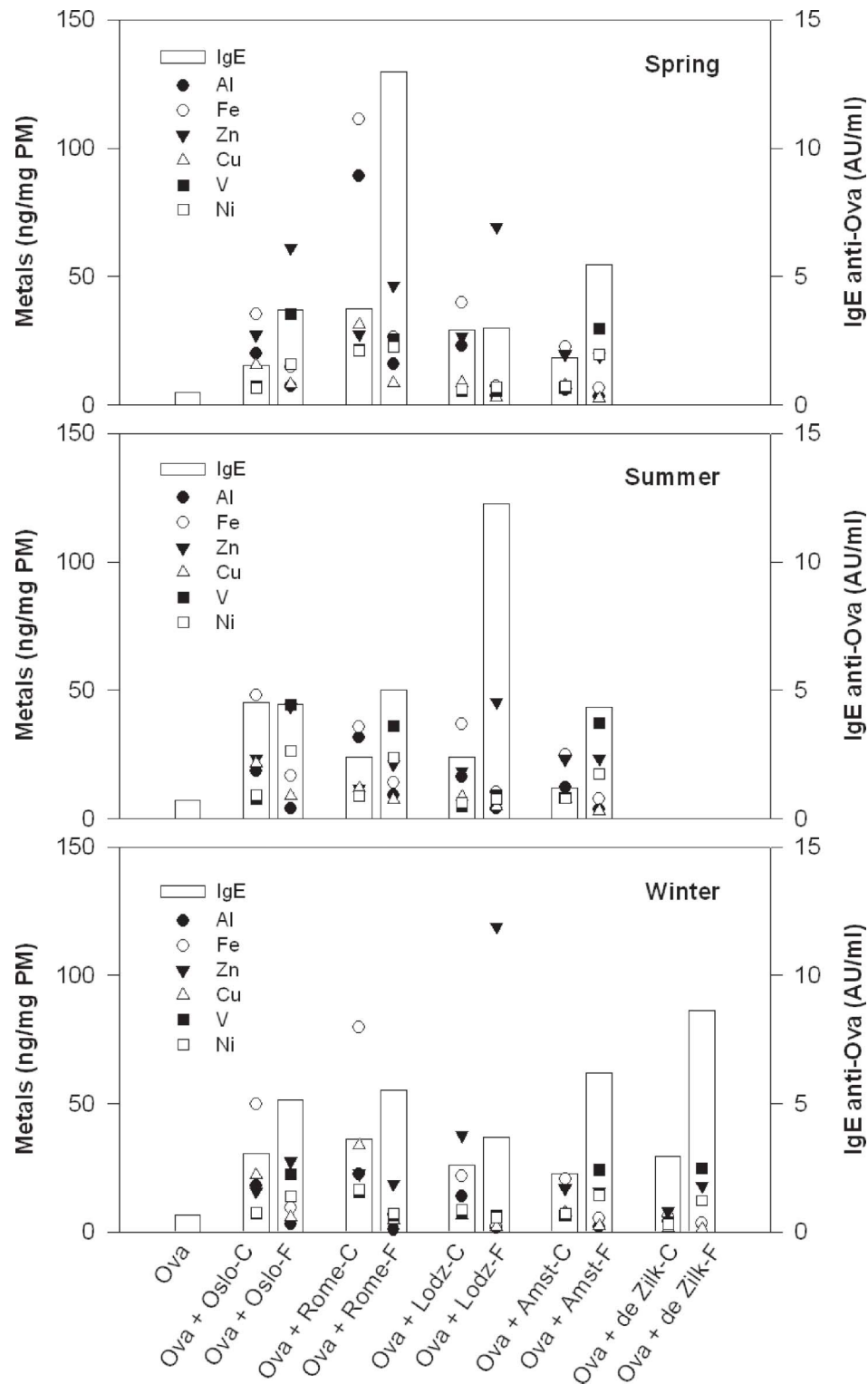


FIG. 4. The concentrations of selected metals in the ambient PM fractions are presented together with the respective PM-induced IgE anti-Ova level. The upper, middle, and lower panels show the results obtained for ambient PM collected in the spring, summer, and winter season, respectively. Points represent the metal concentration, and the bars the median of the IgE anti-Ova level (presented together with the individual results of eight mice in Figure 2). Metal concentration is shown on the left ordinate (ng/mg PM), and IgE anti-Ova levels at the right ordinate (AU/ml). Indicated values in the diagram for Al and Fe should be multiplied by 10^3 , values for Zn and Cu by 10^2 , and values for V and Ni by 10^1 .

in the coarse PM fractions, whereas Zn, V, and Ni often are related to combustion processes and were usually higher in the fine PM fractions. Zn, V, and Ni might therefore be thought to contribute to the higher IgE production associated with the fine compared to the coarse PM fractions, and positive correlations (both correlation coefficients being .6) were observed between V and Ni and the allergen-specific IgE levels when examining all seasons overall. No association was observed between Zn and the levels of allergen-specific IgE. Figure 5 shows the relationship between the concentrations of PAH (light and heavy) in ambient PM and production of allergen-specific IgE. PAHs are found in both size fractions of PM. The highest yields of PAH were found in Lodz, particularly in the winter season. No clear relationship between PAH concentrations and the IgE adjuvant potential of the PM fractions was observed. The association between the inorganic components ammonia (NH_4), potassium (K), nitrate (NO_3), and sulfate (SO_4) of PM and the production of allergen-specific IgE is illustrated in Figure 6. K and NO_3 are found in both the fine and coarse PM fractions. The highest levels of NO_3 were observed for Amsterdam. The summer samples contained high levels of SO_4 , probably due to more warm and humid conditions favorable for more rapid oxidation of sulfur dioxide generated from combustion of fossil fuels. SO_4 and NH_4 are predominantly found in the fine PM fractions, and may therefore contribute to the higher IgE production of fine compared to coarse ambient PM fractions. Although the variation in the content of SO_4 was not reflected in the allergen-specific IgE levels, a positive correlation (with the correlation coefficient being .6) was observed between SO_4 and the production of allergen-specific IgE when examining all seasons overall. It is noteworthy that the PM fractions from the background location de Zilk are not markedly different from the urban locations with regard to the content of metals or to organic or inorganic components.

DISCUSSION

Allergy screening for IgE adjuvant activity showed that in the presence of Ova 12 out of 13 of the fine ambient PM fractions collected nearby busy streets in Oslo, Rome, Lodz, and Amsterdam, in the spring, summer, and winter 2001/2002, exerted a significant IgE adjuvant activity at the dose examined (200 μg single dose per animal). In contrast, only 3 out of 13 of the coarse PM fractions possessed significant adjuvant activity. Overall, fine ambient PM exerted significantly greater IgE adjuvant activity per unit mass than coarse PM. No significant differences were observed between locations or seasons. The finding that PM from the background location de Zilk showed an allergy adjuvant activity similar to that from the four city traffic sites is in agreement with the fact that the PM fractions from de Zilk were chemically equivalent to those from the urban locations. Small combustion particles are transported over long distances, and northwestern winds across the North Sea from British Isles may explain the presence of these particles in the rural samples from de Zilk.

The lack of differences in effects among PM from different locations is probably not due to an insensitivity of the ELISA used, since the assay discriminates between the adjuvant activity of coarse and fine PM, as well as between the Ottawa and European ambient dust. The relatively low IgE anti-Ova response with urban dust from Ottawa, which contains both coarse and fine PM, may be due to a considerable content of large particles or aggregates of particles (observed by transmission electron microscopy analysis, data not shown), which seem to have much less allergy adjuvant activity than the smaller particles.

Epidemiological observations indicate that fine PM exert greater effects with regard to acute asthma-related responses than coarse particles (Penttinen et al., 2001; Schwartz & Neas, 2000). The present findings of a more potent IgE adjuvant effect attributed to fine than coarse PM may suggest that the same or overlapping mechanisms are involved in allergy adjuvant activity and acute precipitation of worsening asthma. Furthermore, our finding that fine PM from ambient air exerts a greater allergy adjuvant effect than coarse PM is in support of the notion that traffic-related air pollution contributes to the disease burden from asthma and allergy (Annesi-Maesano et al., 2007; Janssen et al., 2003; Nicolai et al., 2003), and that traffic pollution not only aggravates preexisting disease but also contributes to the inception and increased prevalence of allergy (Morgenstern et al., 2008). Whether the differences in allergic adjuvant activity between coarse and fine ambient PM fractions can be explained by different elemental composition, adsorbed chemicals, or surface area is not known, and there is evidence for a role of all these factors.

In our study, higher levels of specific components of PM such as SO_4 , NH_4 , V, Ni, and Zn were present in the fine compared to the coarse PM fractions, and these components might therefore be thought to contribute to the higher IgE production associated with the fine compared to coarse PM fractions. However, differences in the content of SO_4 and Zn among fine PM fractions did not reflect the variation in levels of IgE anti-Ova. Still, when comparing all seasons overall positive correlations were observed between V, Ni, and SO_4 and the allergen specific IgE responses. The finding that V and Ni are associated with the production of allergen specific IgE is in accordance with allergic diseases being high at locations in Germany characterized by high levels of metals such as lead (Pb), cadmium (Cd), chromium (Cr), and Ni in ambient air (Heinrich et al., 1999; Wilhelm et al., 2007). Further, the metal composition of ambient air was found to influence the severity of allergic respiratory disease in a mouse model (Gavett et al., 2003). Ni and V were also shown to enhance allergic sensitization to house dust mite in a rat model (Lambert et al., 2000). SO_4 , on the other hand, in epidemiologic studies primarily was associated with bronchitis, not with allergy and asthma (Dockery et al., 1996; von Mutius et al., 1992). Moreover, the concentration of SO_4 in Asian sand dust did not reflect the degree of eosinophilic lung inflammation produced by Asian sand dust and Ova (Hiyoshi

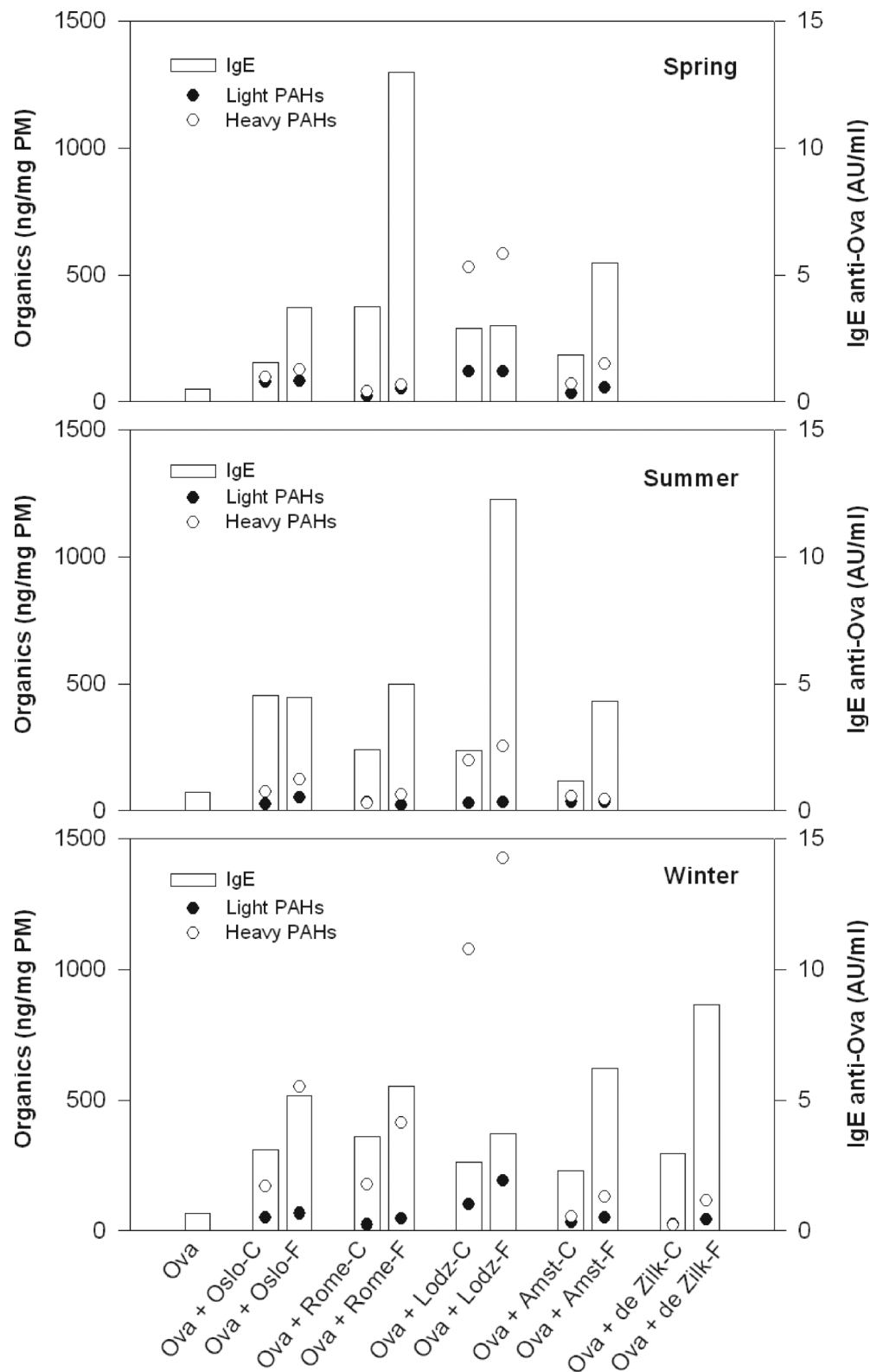


FIG. 5. The concentration of PAHs (light and heavy) in the ambient PM fractions presented with the respective PM-induced IgE anti-Ova level. The upper, middle, and lower panels show the results obtained for ambient PM collected in the spring, summer, and winter season, respectively. Points represent the PAH concentrations, and bars the median of the IgE anti-Ova level (presented together with the individual results of 8 mice in Figure 2). The PAH concentration is shown on the left ordinate (ng/mg PM), and IgE anti-Ova levels at the right ordinate (AU/ml).

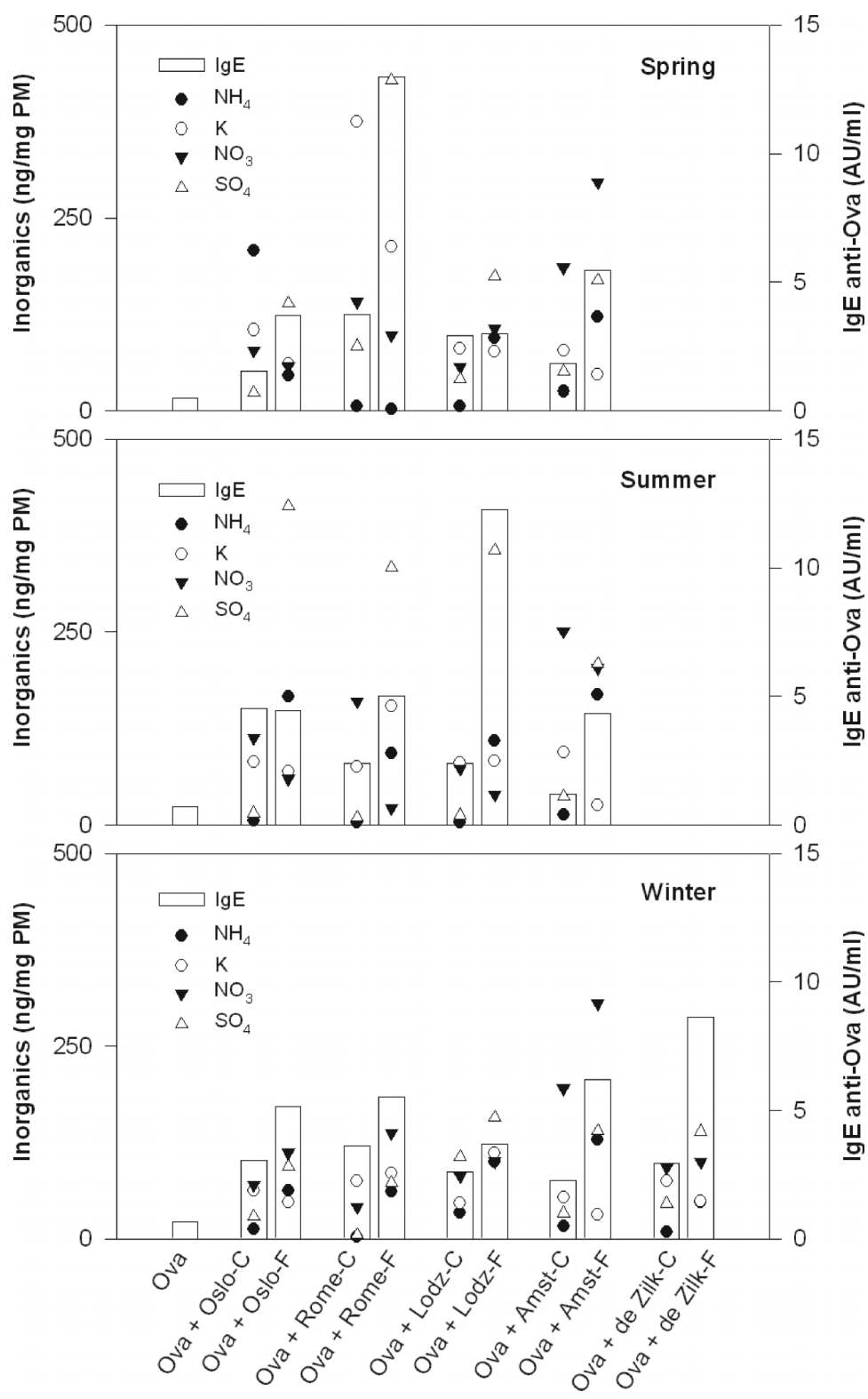


FIG. 6. The concentration of selected inorganic components in the ambient PM fractions presented together with the respective PM-induced IgE anti-Ova level. The upper, middle, and lower panels show the results obtained for ambient PM collected in the spring, summer, and winter season, respectively. Points represent the concentration of inorganic components, and bars the median of the IgE anti-Ova level (presented together with the individual results of eight mice in Figure 2). The concentration of inorganic components is shown on the left ordinate (ng/mg PM), and IgE anti-Ova levels at the right ordinate (AU/ml). Indicated values in the diagram for K should be multiplied by 10².

et al., 2005). However, in those studies only Ova and Asian sand dust containing high levels of SO₄ stimulated the production of allergen-specific IgG1. No association was found between the PAH levels in the ambient PM and the allergen-specific IgE responses. Our findings are in agreement with the epidemiological observation that allergic diseases are not associated with high levels of PAH at a location in Germany (Wilhelm et al., 2007). The evidence for a role of PAH in allergic diseases, however, is conflicting. In studies in vitro, PAHs extracted from DEP enhanced ongoing IgE production in human B cells (Takenaka et al., 1995) and induced histamine release from a murine mast cell line (Diaz-Sanchez et al., 2000). Organic extracts from urban PM also seem to be of importance in the induction of allergic diseases by activating human basophils (Devouassoux et al., 2002; Schober et al., 2007). Moreover, PAH in the RAIAP ambient PM were associated with the production of allergen-specific IgE after intranasal exposure (whereas V, Ni, and SO₄ were not) (Steerenberg et al., 2006). The conflicting results for PAH may to some extent be explained by the fact that in some studies the effect of soluble PAH were examined, whereas in other studies the effect of PM associated PAH were determined.

Particle size appears to be of importance since fine but not coarse polystyrene particles possess allergy adjuvant activity (Nygaard et al., 2004). Moreover, fine PM has been reported to exert an allergy adjuvant effect regardless of composition (Granum et al., 2001). Allergy adjuvant activity of polystyrene particles in mice was shown to be determined by particle number and surface area, and not particle mass (Nygaard et al., 2004). Small particles have per mass unit much larger numbers and surface area than larger particles. A large surface area is advantageous if particles exert their adjuvant activity by acting as a carrier of allergen, or as a carrier of chemicals and metals, or by other surface interactions. By acting as a carrier of allergen, particles may make the uptake, processing, and presentation in antigen-presenting cells more effective.

The quality of an immune response (Th1 or Th2) to an antigen in mice is dependent on the adjuvant used (van Zijverden et al., 2000), the antigen characteristics (Dearman et al., 2000), dose and frequency of exposure (Kolbe et al., 1994; Morokata et al., 2000; Nelde et al., 2001), site of delivery (Constant et al., 2000; Nelde et al., 2001; Repa et al., 2004), and animal strain, i.e., genetic factors (Brewer et al., 1999; Constant et al., 2000; Nygaard et al., 2005). Although the route of exposure is of importance, an adjuvant effect of particles on the allergen specific IgE production was demonstrated in mice regardless of the route of exposure (Granum et al., 2001; Lovik et al., 1997; Muranaka et al., 1986; Takano et al., 1997). The allergy adjuvant activity of the RAIAP PM observed following sc exposure in the footpads is therefore assumed to be of importance also following exposure via routes more relevant for the real-life situation, specifically inhalation exposure. This notion is supported by the demonstration of an allergy adjuvant effect of the ambient RAIAP PM following intranasal bolus exposure

(Steerenberg et al., 2004, 2005). Although discrepancies were found with regard to which PM fractions that responded, the fine PM fractions in both mouse models (using different routes of exposure) induced a greater IgE immune response than coarse PM fractions. This fact strengthens the value of the IgE footpad immunization model as a screening model for adjuvant activity of air pollutants.

The PLN assay, on the other hand, appears not to be useful as a quantitative assay for screening of allergy adjuvant activity, although the weight and cellular responses to particles and Ova in combination were significantly greater than to Ova or particles alone. Moreover, the response turned out to be synergistic, not just an additive effect of Ova and particles. However, there was no correlation between the level of allergen-specific IgE production and the PLN weight and cell number responses. This may be due to the fact that the PLN assay does not distinguish between an allergic and a nonallergic immune response, or a nonspecific inflammatory response. The potent adjuvant activity of the fine compared to coarse PM fractions observed in the allergen-specific IgE assay was not reflected in the PLN assay, which indicates better relevance and sensitivity of the IgE assay.

In conclusion, compared to the PLN assay, the allergen-specific IgE footpad immunization assay is clearly to be preferred for screening for allergy adjuvant activity of PM. Fine PM fractions consistently were found to increase the allergen-specific IgE responses more than the coarse ones. Our finding is in agreement with the notion that traffic-related air pollution contributes to the disease burden in asthma and allergy, and points to fine and ultrafine ambient air PM as the most important fractions in relation to allergic diseases.

REFERENCES

- Aalto, P., Hameri, K., Paatero, P., Kulmala, M., Bellander, T., Berglund, N., Bouso, L., Castano-Vinyals, G., Sunyer, J., Cattani, G., Marconi, A., Cyrys, J., von Klot, S., Peters, A., Zetzsche, K., Lanki, T., Pekkanen, J., Nyberg, F., Sjoval, B., and Forastiere, F. 2005. Aerosol particle number concentration measurements in five European cities using TSI-3022 condensation particle counter over a three-year period during health effects of air pollution on susceptible subpopulations. *J. Air Waste Manage. Assoc.* 55:1064–1076.
- Annesi-Maesano, I., Moreau, D., Caillaud, D., Lavaud, F., Le Moullec, Y., Taytard, A., Pauli, G., and Charpin, D. 2007. Residential proximity fine particles related to allergic sensitisation and asthma in primary school children. *Respir. Med.* 101:1721–1729.
- Braback, L., Breborowicz, A., Dreborg, S., Knutsson, A., Pieklik, H., and Bjorksten, B. 1994. Atopic sensitization and respiratory symptoms among Polish and Swedish school children. *Clin. Exp. Allergy* 24:826–835.
- Brewer, J. P., Kisselgof, A. B., and Martin, T. R. 1999. Genetic variability in pulmonary physiological, cellular, and antibody responses to antigen in mice. *Am. J. Respir. Crit Care Med.* 160:1150–1156.
- Cassee, F. R., Fokkens, P. H. B., Leseman, D. L. A. C., Bloemen, H. J. T., and Boere, A. J. F. 2003. *Respiratory allergy and inflammation due to ambient particles (RAIAP). Collection and characterisation of particulate matter samples from five European sites.* RIVM report, <http://www.rivm.nl/bibliotheek/rapporten/863001001.pdf>
- Constant, S. L., Lee, K. S., and Bottomly, K. 2000. Site of antigen delivery can influence T cell priming: Pulmonary environment promotes preferential Th2-type differentiation. *Eur. J. Immunol.* 30:840–847.

- D'Amato, G., Liccardi, G., D'Amato, M., and Holgate, S. 2005. Environmental risk factors and allergic bronchial asthma. *Clin. Exp. Allergy*. 35:1113–1124.
- Dearman, R. J., Caddick, H., Basketter, D. A., and Kimber, I. 2000. Divergent antibody isotype responses induced in mice by systemic exposure to proteins: A comparison of ovalbumin with bovine serum albumin. *Food Chem. Toxicol.* 38:351–360.
- Demokritou P., Kavouras I. G., Ferguson S. T., and Koutrakis, P. 2002. Development of a high volume cascade impactor for toxicological and chemical characterization. *Aerosol Sci. Technol.* 36:925–933.
- Devouassoux, G., Saxon, A., Metcalfe, D. D., Prussin, C., Colomb, M. G., Brambilla, C., and Diaz-Sanchez, D. 2002. Chemical constituents of diesel exhaust particles induce IL-4 production and histamine release by human basophils. *J. Allergy Clin. Immunol.* 109:847–853.
- Diaz-Sanchez, D., Penichet-Garcia, M., and Saxon, A. 2000. Diesel exhaust particles directly induce activated mast cells to degranulate and increase histamine levels and symptom severity. *J. Allergy Clin. Immunol.* 106:1140–1146.
- Dockery, D. W., Cunningham, J., Damokosh, A. I., Neas, L. M., Spengler, J. D., Koutrakis, P., Ware, J. H., Raizenne, M., and Speizer, F. E. 1996. Health effects of acid aerosols on North American children: Respiratory symptoms. *Environ. Health Perspect.* 104:500–505.
- Dybing, E., Lovdal, T., Hetland, R. B., Lovik, M., and Schwarze, P. E. 2004. Respiratory allergy adjuvant and inflammatory effects of urban ambient particles. *Toxicology* 198:307–314.
- Ebel, S., Brauer, M., Cyrys, J., Tuch, T., Kreyling, W. G., Wichmann, H. E., and Heinrich, J. 2001. Air quality in postunification Erfurt, East Germany: Associating changes in pollutant concentrations with changes in emissions. *Environ. Health Perspect.* 109:325–333.
- Gavett, S. H., Haykal-Coates, N., Copeland, L. B., Heinrich, J., and Gilmour, M. I. 2003. Metal composition of ambient PM_{2.5} influences severity of allergic airways disease in mice. *Environ. Health Perspect.* 111:1471–1477.
- Gilmour, M. I., Jaakkola, M. S., London, S. J., Nel, A. E., and Rogers, C. A. 2006. How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. *Environ. Health Perspect.* 114:627–633.
- Granum, B., Gaarder, P. I., Groeng, E., Leikvold, R., Namork, E., and Lovik, M. 2001. Fine particles of widely different composition have an adjuvant effect on the production of allergen-specific antibodies. *Toxicol. Lett.* 115:171–181.
- Harrison, R. M., and Jones, A. M. 2005. Multisite study of particle number concentrations in urban air. *Environ. Sci. Technol.* 39:6063–6070.
- Heinrich, J., Hoelscher, B., Wjst, M., Ritz, B., Cyrys, J., and Wichmann, H. 1999. Respiratory diseases and allergies in two polluted areas in East Germany. *Environ. Health Perspect.* 107:53–62.
- Hiyoshi, K., Ichinose, T., Sadakane, K., Takano, H., Nishikawa, M., Mori, I., Yanagisawa, R., Yoshida, S., Kumagai, Y., Tomura, S., and Shibamoto, T. 2005. Asian sand dust enhances ovalbumin-induced eosinophil recruitment in the alveoli and airway of mice. *Environ. Res.* 99:361–368.
- ISAAC and the International Study of Asthma and Allergies in Childhood Steering Committee. 1998. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet* 351:1225–1232.
- Janson, C., Anto, J., Burney, P., Chinn, S., de Marco, R., Heinrich, J., Jarvis, D., Kuenzli, N., Leynaert, B., Luczynska, C., Neukirch, F., Svanes, C., Sunyer, J., and Wjst, M. 2001. The European Community Respiratory Health Survey: What are the main results so far? European Community Respiratory Health Survey II. *Eur. Respir. J.* 18:598–611.
- Janssen, N. A., Brunekreef, B., Van Vliet, P., Aarts, F., Meliefste, K., Harssema, H., and Fischer, P. 2003. The relationship between air pollution from heavy traffic and allergic sensitization, bronchial hyperresponsiveness, and respiratory symptoms in Dutch schoolchildren. *Environ. Health Perspect.* 111:1512–1518.
- Kolbe, L., Heusser, C., and Kolsch, E. 1994. Epitope-dependent nonreciprocal regulation of IgE and IgG2a antibody formation. *Int. Arch. Allergy Immunol.* 103:214–216.
- Kreyling, W. G., Tuch, T., Peters, A., Pitz, M., Heinrich, J., Stolzel, M., Cyrys, J., Heyder, J., and Wichmann, H. E. 2003. Diverging long-term trends in ambient urban particle mass and number concentrations associated with emission changes caused by the German unification. *Atmos. Environ.* 37:3841–3848.
- Lambert, A. L., Dong, W., Selgrade, M. K., and Gilmour, M. I. 2000. Enhanced allergic sensitization by residual oil fly ash particles is mediated by soluble metal constituents. *Toxicol. Appl. Pharmacol.* 165:84–93.
- Lovik, M., Hogseth, A. K., Gaarder, P. I., Hagemann, R., and Eide, I. 1997. Diesel exhaust particles and carbon black have adjuvant activity on the local lymph node response and systemic IgE production to ovalbumin. *Toxicology* 121:165–178.
- Morgenstern, V., Zutavern, A., Cyrys, J., Brockow, I., Koletzko, S., Kramer, U., Behrendt, H., Herbarth, O., von Berg, A., Bauer, C. P., Wichmann, H. E., and Heinrich, J. 2008. Atopic diseases, allergic sensitization, and exposure to traffic-related air pollution in children. *Am. J. Respir. Crit. Care Med.* 177:1331–1337.
- Morokata, T., Ishikawa, J., and Yamada, T. 2000. Antigen dose defines T helper 1 and T helper 2 responses in the lungs of C57BL/6 and BALB/c mice independently of splenic responses. *Immunol. Lett.* 72:119–126.
- Muranaka, M., Suzuki, S., Koizumi, K., Takafuji, S., Miyamoto, T., Ikemori, R., and Tokiwa, H. 1986. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. *J. Allergy Clin. Immunol.* 77:616–623.
- Namork, E., Kurup, V. P., Aasvang, G. M., and Johansen, B. V. 2004. Detection of latex allergens by immunoelectron microscopy in ambient air (PM₁₀) in Oslo, Norway (1997–2003). *J. Environ. Health* 67:20–26.
- Nel, A., Teufel, M., Hahn, C., Duschl, A., Sebald, W., Brocker, E. B., and Grunewald, S. M. 2001. The impact of the route and frequency of antigen exposure on the IgE response in allergy. *Int. Arch. Allergy Immunol.* 124:461–469.
- Nicolai, T., Carr, D., Weiland, S. K., Duhme, H., von Ehrenstein, O., Wagner, C., and von Mutius, E. 2003. Urban traffic and pollutant exposure related to respiratory outcomes and atopy in a large sample of children. *Eur. Respir. J.* 21:956–963.
- Nygaard, U. C., Aase, A., and Lovik, M. 2005. The allergy adjuvant effect of particles—Genetic factors influence antibody and cytokine responses. *BMC Immunol.* 6:11.
- Nygaard, U. C., Samuelsen, M., Aase, A., and Lovik, M. 2004. The capacity of particles to increase allergic sensitization is predicted by particle number and surface area, not by particle mass. *Toxicol. Sci.* 82:515–524.
- Ormstad, H., Gaarder, P. I., Johansen, B. V., and Lovik, M. 1998. Airborne house dust elicits a local lymph node reaction and has an adjuvant effect on specific IgE production in the mouse. *Toxicology* 129:227–236.
- Paoletti, L., De Berardis, B., and Diociaiuti, M. 2002. Physico-chemical characterisation of the inhalable particulate matter (PM₁₀) in an urban area: An analysis of the seasonal trend. *Sci. Total Environ.* 292:265–275.
- Penttinen, P., Timonen, K. L., Tiittanen, P., Mirmé, A., Ruuskanen, J., and Pekkanen, J. 2001. Ultrafine particles in urban air and respiratory health among adult asthmatics. *Eur. Respir. J.* 17:428–435.
- Repa, A., Wild, C., Hufnagl, K., Winkler, B., Bohle, B., Pollak, A., and Wiedermann, U. 2004. Influence of the route of sensitization on local and systemic immune responses in a murine model of type I allergy. *Clin. Exp. Immunol.* 137:12–18.
- Schober, W., Lubitz, S., Belloni, B., Gebauer, G., Lintemann, J., Matuschek, G., Weichenmeier, I., Eberlein-König, B., Buters, J., and Behrendt, H. 2007. Environmental polycyclic aromatic hydrocarbons (PAHs) enhance allergic inflammation by acting on human basophils. *Inhal. Toxicol.* 19(Suppl. 1):151–156.
- Schwartz, J., and Neas, L. M. 2000. Fine particles are more strongly associated than coarse particles with acute respiratory health effects in schoolchildren. *Epidemiology* 11:6–10.
- Sillanpää, M., Hillamo R., Makela T., Pennanen A.S., and Salonen, R. O. 2003. Field and laboratory tests of a high volume cascade impactor. *J. Aerosol Sci.* 34:485–500.
- Steenenbergh, P. A., van Amelsvoort, L., Lovik, M., Hetland, R. B., Alberg, T., Halatek, T., Bloemen, H. J., Rydzynski, K., Swaen, G., Schwarze, P., Dybing, E., and Cassee, F. R. 2006. Relation between sources of particulate air pollution and biological effect parameters in samples from four European cities: An exploratory study. *Inhal. Toxicol.* 18:333–346.
- Steenenbergh, P. A., Withagen, C. E., van Dalen, W. J., Dormans, J. A., Cassee, F. R., Heisterkamp, S. H., and van Loveren, H. 2004. Adjuvant activity of ambient particulate matter of different sites, sizes, and seasons in a respiratory allergy mouse model. *Toxicol. Appl. Pharmacol.* 200:186–200.

- Steerenberg, P. A., Withagen, C. E. T., van Dalen, W. J., Dormans, J. A. M. A., Heisterkamp, S. H., van Loveren, H., and Cassee, F. R. 2005. Dose dependency of adjuvant activity of particulate matter from five European sites in three seasons in an ovalbumin-mouse model. *Inhal. Toxicol.* 17:133–145.
- Takano, H., Yoshikawa, T., Ichinose, T., Miyabara, Y., Imaoka, K., and Sagai, M. 1997. Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. *Am. J. Respir. Crit. Care Med.* 156:36–42.
- Takenaka, H., Zhang, K., Diaz-Sanchez, D., Tsien, A., and Saxon, A. 1995. Enhanced human IgE production results from exposure to the aromatic hydrocarbons from diesel exhaust: Direct effects on B-cell IgE production. *J. Allergy Clin. Immunol.* 95:103–115.
- van Zijverden, M., van der Pijl, A., Bol, M., van Pinxteren, F. A., de Haar, C., Penninks, A. H., van Loveren, H., and Pieters, R. 2000. Diesel exhaust, carbon black, and silica particles display distinct Th1/Th2 modulating activity. *Toxicol. Appl. Pharmacol.* 168:131–139.
- von Mutius, E., Fritzsche, C., Weiland, S. K., Roll, G., and Magnussen, H. 1992. Prevalence of asthma and allergic disorders among children in united Germany: A descriptive comparison. *Br. Med. J.* 305:1395–1399.
- von Mutius, E., Martinez, F. D., Fritzsche, C., Nicolai, T., Roell, G., and Thiemann, H. H. 1994. Prevalence of asthma and atopy in two areas of West and East Germany. *Am. J. Respir. Crit. Care Med.* 149:358–364.
- von Mutius, E., Weiland, S. K., Fritzsche, C., Duhme, H., and Keil, U. 1998. Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany. *Lancet* 351:862–866.
- Weiland, S. K., von Mutius, E., Hirsch, T., Duhme, H., Fritzsche, C., Werner, B., Husing, A., Stender, M., Renz, H., Leupold, W., and Keil, U. 1999. Prevalence of respiratory and atopic disorders among children in the East and West of Germany five years after unification. *Eur. Respir. J.* 14:862–870.
- Wilhelm, M., Eberwein, G., Holzer, J., Gladtko, D., Angerer, J., Marczynski, B., Behrendt, H., Ring, J., Sugiri, D., and Ranft, U. 2007. Influence of industrial sources on children's health—Hot spot studies in North Rhine Westphalia, Germany. *Int. J. Hyg. Environ. Health* 210:591–599.