



Detection of allergens adsorbed to ambient air particles collected in four European cities

Ellen Namork^{a,*}, Bjørn V. Johansen^b, Martinus Løvik^{a,c}

^a Norwegian Institute of Public Health, P.O. Box 4404 Nydalen, NO-0403 Oslo, Norway

^b Department of Molecular Biosciences, University of Oslo, Norway

^c Norwegian University of Science and Technology, Trondheim, Norway

Received 30 November 2005; received in revised form 23 January 2006; accepted 24 January 2006

Abstract

Air pollution has been implicated as one of the factors responsible for the increased incidence of allergic diseases observed in recent years. High concentrations of air pollutants may promote airway sensitization by acting as adjuvants. Ambient particles as carriers of adsorbed allergens are, therefore, of special interest since they may act as mediators of inflammatory as well as allergic responses. Ambient air particles from four cities in Europe were collected, in three different seasons, to examine the variation of allergens and their possible binding to the pollution particles. The particle fraction, PM₁₀, was collected on polycarbonate filters using a low-volume sampling regime. The presence of pollen allergens, latex and β -glucans was investigated using an immunogold labelling method directly on the collection filters. Scanning electron microscopy revealed mainly the classical carbon particles and aggregates determined to originate from vehicle exhaust. The immunogold labelling visualised in the backscatter electron imaging mode, showed that allergens from pollens, latex and also β -glucans were bound to and, hence, transported by the combustion particles in ambient air. Thus, combustion particles in ambient air are carriers of allergens and act as depots of allergens inhaled into the airways.

© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: Air pollution; Combustion particles; Outdoor allergens; Allergy; Scanning electron microscopy

1. Introduction

Environmental factors such as allergens and air pollution have been proposed to promote the induction and elicitation of respiratory allergies. It has been demonstrated that pollen allergens from trees, grasses and weeds are released in the form of submicronic particles that may be loaded onto other particles (Grote et al., 2003; Taylor et al., 2004). Correspondingly, combustion

particles from vehicles and industry and their aggregates in ambient air, have been shown to adsorb allergens (Knox et al., 1997; Namork et al., 2004; Ormstad et al., 1995). Furthermore, combustion particles have an adjuvant effect increasing the IgE production of susceptible individuals upon exposure to allergens (Diaz-Sanchez et al., 1999; Nygaard et al., 2005; Takafuji et al., 1989a). Thus, the interaction between air pollution particles and allergens may enhance allergic airway responses (D'Amato, 2002; Polosa, 2001).

The relation between particle exposure and adverse health effects shows geographical differences (Janson et al., 2001). Ambient particles may differ qualitatively at different locations, accounting for differences

* Corresponding author. Tel.: +47 22042433; fax: +47 22042686.

E-mail addresses: ellen.namork@fhi.no (E. Namork),
b.v.johansen@imbv.uio.no (B.V. Johansen),
martinus.lovik@fhi.no (M. Løvik).

in relation to the induction of inflammatory responses and the elicitation of allergy (Polosa et al., 2002). A research project “Respiratory Allergy and Inflammation due to Ambient Particles—a European-wide Assessment” (RAIAP, 2004), was initiated to investigate if qualitative differences in particulate air pollution partly could explain differences in prevalence and severity of respiratory allergies throughout Europe (Nygaard et al., 2005). Ambient air particle fractions were collected in the cities of Amsterdam in The Netherlands, Lodz in Poland, Rome in Italy and Oslo in Norway, during the spring, winter and summer seasons in 2001–2002.

The specific object of the present study was to examine ambient air samples from cities in the western, central, northern and southern Europe, for representative pollen allergens carried by ambient particles. Since vehicle traffic is a predominant particle source in urban areas, and latex is a substance in car tires, the presence of latex allergens was also investigated. Beta-(1-3)-glucans, from the cell wall of plants and microbes, are also believed to play a role in bioaerosol-induced respiratory symptoms (Wan et al., 1999; Rylander and Lin, 2000). An immunogold labelling method was used to detect the allergens and β -glucans, and the labelling was visualised in the backscatter electron imaging mode of a scanning electron microscope (Namork, 1991).

2. Materials and methods

2.1. Sampling of particulate matter

A high volume cascade impactor was used to collect particulate matter (PM) fractions, in spring, summer and winter in the cities of Amsterdam, Rome, Lodz and Oslo for physicochemical characterization (Cassee et al., 2003). The duration of sampling and transportation of the impactor between the countries in the different seasons allowed only one sampling at each site per season. Low volume sampling employing a commercially available impactor unit for collection of PM₁₀ (URG Corp. USA) was built into the housing of the high volume cascade impactor to collect ambient air particles, at the same sites and times, for electron microscopy. The low volume sampler was equipped with a timer and a flow controller. Ambient particles were collected on 0.4 μ m polycarbonate filters (25 mm ϕ), using a flow of 2 l/min. The sampler was run intermittently at pre-set intervals for 2 weeks to get representative particles over a period of time and with a particle load suitable for electron microscopy. The cutpoint of the PM₁₀ impactor running at 2 l/min was 10 μ m. The filters were equilibrated, in room temperature, for 24 h and weighed, both before and after collection, using a microbalance (Mettler, MT5). The three seasons were selected aiming at the collection of PM₁₀ from traffic and the different pollen allergens of interest in spring and summer, and latex allergens possibly in all seasons. Representative ambient

air samples were collected at locations dominated by traffic emission, 10–30 m from heavy traffic in all four major cities (for precise description of sampling framework and locations see Cassee et al., 2003).

2.2. Immunogold labelling

The immunogold labelling was performed directly on the collection filters using the appropriate primary antibodies and secondary antibodies conjugated to a colloidal gold sphere of 30 nm (gold probe). 3 mm ϕ disks of the collection filters were punched out and incubated, collection side down, on a drop (10 μ l) of the primary antibody. The specimens were washed on several drops of buffer and incubated on a drop containing the gold probe. After washing, the specimens were mounted on stubs using carbon tape and coated, by evaporation of graphite rods, with a thin (<10 nm) layer of carbon. The buffer used throughout the investigation was 0.1 M ammonium acetate, pH 7.0. All monoclonal antibodies were diluted to approximately 3 mg/ml and the polyclonal antibodies to 1:500 and 1:1000 in buffer. Appropriate positive and negative controls were included for each antibody.

2.3. Antibodies

Most of the antibodies used were kindly given to us. The donor names and references to the producers of the antibodies are given in brackets. All filters, from all cities and seasons, were labelled with a monoclonal antibody (LAM-2) produced against the 23-kDa latex polypeptide, *Hev b 3* (Kurup, V.P., Wisconsin, USA, Lu et al., 1995) and a polyclonal antibody to β -glucans (Doekes, G., Utrecht, The Netherlands, Douwes et al., 1996). A monoclonal antibody (Bo 1) to timothy grass pollen, *Phl p 1* (Petersen, A., Borstel, Germany, Petersen et al., 1995) was applied to all filters except Rome. Three different monoclonal antibodies to the main allergen of birch denoted *Bet v 1* (clone M82/D3/C8, Smith Kline Beecham, London, England), BIP1 (Jensen-Jarolim, E., Vienna, Austria, Jensen-Jarolim et al., 1999), and mP16 (Wiche, R., Langen, Germany, Wiche et al., 2005), and a polyclonal antibody to birch, anti-*Bet v* (purchased from Pharmacia Upjohn, Sweden) were applied to filters from Oslo, Lodz and the spring filter from Amsterdam. Monoclonal antibodies to pellitory-on-the-wall, *Par j 1* (pj 6), and olive, *Ole e 1* (Ol 7) (Duffort, O., ALK-Abello, Spain (Ayuso et al., 1993; Lombardero et al., 1992, respectively) were used on all filters from Rome. All monoclonal antibodies were made from mouse cells and the two polyclonal antibodies from rabbit serum. The gold probes applied were goat anti-mouse IgG-30 nm and goat anti-rabbit IgG-30 nm (BioCell Research Laboratories, Cardiff, UK).

2.4. Controls

Pollen extracts (birch (No. 108), timothy (No. 225), pellitory-on-the-wall (No. 233) and olive (No. 224)) used for positive controls (purchased from ALK-Abello, Copenhagen,

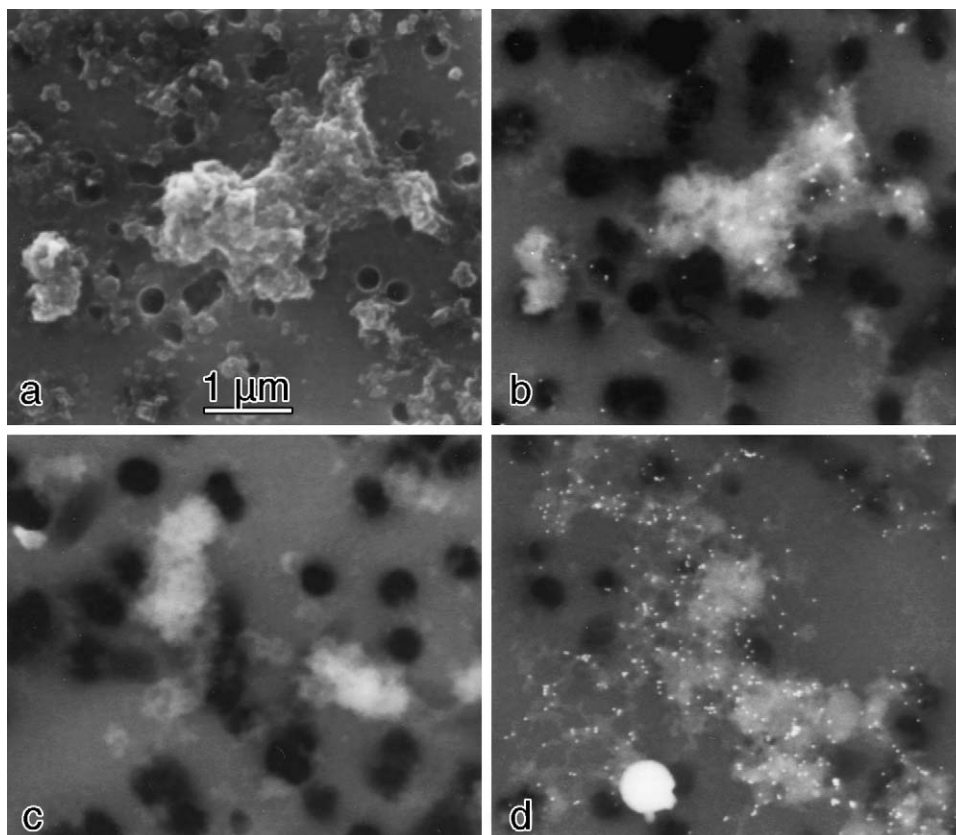


Fig. 1. Scanning electron micrographs of ambient air particulate matter (PM₁₀), collected on filters and immunogold labelled. Micrographs (a) and (b) show the same carbon aggregate labelled with anti-*Phl p 1* to timothy: (a) imaged with secondary electrons to enable observation of the morphology, and (b) with backscatter electrons to visualize the immunogold labelling. Note that the gold particles (white spots) label carbon particles and aggregates only, and not the filter background. A negative and positive control are shown in (c) and (d) using irrelevant (IgG) primary antibody and timothy extract (No. 228), respectively.

Denmark) were incubated on the respective filters and immunogold labelled as above (example in Fig. 1d). As positive control for anti-*Hev b 3*, a latex glove (Safeskin Corp., Boca Raton, Florida, USA), and for β -glucan from yeast, MacroGuard[®] Sol 2% (BioTec, Tromsø, Norway) were used. The negative controls were buffer and isotype controls from mouse and normal rabbit serum, replacing the primary antibody (Fig. 1c).

2.5. Scanning electron microscopy

The morphology of the ambient particles was observed with secondary electrons (Figs. 1a and 2a,c,e), while the gold particles were visualised as white spots in the backscatter electron imaging (BEI) mode (Figs. 1b,d and 2b,d,f). The scanning electron microscope (JSM 840, Jeol Ltd., Japan) was operated at 15 KeV, with a current of 1×10^{-10} A and a working distance of 7 mm. To differentiate between the immunolabelling density on the particle aggregates on the different filters, semi-quantitative recording was performed as no labelling (–), weak (+), moderate (++) and strong (+++) labelling (Table 1) using the strongest labelling of the positive controls as reference for (+++) (example in Fig. 1d). Particle aggregates labelled with less than 10

gold spheres were characterized as (–), more than 10 as weakly labelled (+), while moderately labelled (++) particle aggregates were judged with reference to the strongly and weakly labelled ones. The strength of labelling was judged during microscopy of at least 10 different areas on each specimen. Similar methods of semi-quantification has been used in other studies (Namork, 1991; Namork et al., 2004; Ormstad et al., 1997; Soligo et al., 1985).

3. Results

The mass concentrations of ambient air particles were higher in winter for all four cities, compared to the other two seasons. Using the low volume collection method, the highest mass concentrations were measured in winter in Lodz (66 $\mu\text{g}/\text{m}^3$) and Rome (61 $\mu\text{g}/\text{m}^3$) compared to 34 $\mu\text{g}/\text{m}^3$ and 13 $\mu\text{g}/\text{m}^3$ in Oslo and Amsterdam, respectively. The mass concentrations were lowest in spring in Oslo (15 $\mu\text{g}/\text{m}^3$) and Amsterdam (16.3 $\mu\text{g}/\text{m}^3$) compared to Lodz (22.9 $\mu\text{g}/\text{m}^3$) and Rome (33.8 $\mu\text{g}/\text{m}^3$).

Table 1

Labelling density of carbon aggregates (PM₁₀) on the collection filters from all three seasons in the four cities, Lodz, Oslo, Amsterdam and Rome

2001–2003	PoAb anti- <i>Bet v</i>	Ctr. allergen (No. 108)	MoAb anti- <i>Phl p1</i>	Ctr. allergen (No. 225)	PoAb anti-β- glucan	Ctr. Macro- Guard	MoAb anti- <i>Hev b3</i>	Ctr. latex glove
Lodz								
Spring	—	++	—	++	— +++*	+++	+	+++
Summer	— ++*	++	—	++	— +++*	+++	+	+++
Winter	—	++	(+)	++	— ++*	+++	+	+++
Oslo								
Spring	—	+++	+(+)	++	No filter available		+	+++
Summer	— ++*	+++	+(+)	++	— +++*	+++	+	+++
Winter	—	+++	+	++	— ++*	+++	+	+++
Amsterdam								
Spring ^a	— +*	+	—	++	— +++*	+++	—	+++
Summer	Not done	Not done	(+)	++	— +++*	+++	+	+++
Winter ^b	Not done	Not done	(+)	++	—	+++	+	+++
	MoAb anti- <i>Par j1</i>	Ctr. allergen (No. 233)	MoAb anti- <i>Ole e1</i>	Ctr. allergen (No. 224)				
Rome								
Spring	— ++*	++	+ +*	++	+ +++*	+++	+	+++
Summer	+ +*	++	+ +*	++	— ++*	+++	+	+++
Winter	+ +*	++ ++*	+ +*	++	++ +++*	+++	+	+++

See text, Section 3, for further explanations. MoAb: monoclonal antibody; PoAb: polyclonal antibody; Ctr.: control.

^a Heavy rain during sampling.

^b Very little material on the filter.

* Labelling density of organic/plant material.

Scanning electron microscopy (SEM) showed that all filters irrespective of season, contained mainly carbon particles and aggregates of different sizes up to 10 μm, that were immunogold labelled using the present antibodies (Figs. 1 and 2). Some particles of different morphology were judged to be of vegetal origin, because of their smooth morphology in contrast to the grape-like clusters of combustion particles, and by their low electron density as opposed to mineral particles. The semi-quantitative results of the immunogold labelling of the filters from the different seasons and cities are shown in Table 1. No labelling was observed using any of the three monoclonal antibodies to the birch pollen allergen, *Bet v 1*, on any of the filters examined or on the positive control (No. 108) (not shown). In contrast, the polyclonal antibody to birch allergens bound to the carbon particles and aggregates in the positive control.

On the collected filters, however, some plant materials were seen to be labelled (marked with an asterisk, Table 1), but not the carbon particles. The antibody to the timothy grass pollen allergen, bound weakly to the carbon aggregates on the filters from all seasons in Oslo (Fig. 1b). Some gold particles ((+) in Table 1) could also be observed labelling the carbon aggregates in the winter filter from Lodz, and the summer and winter filters from Amsterdam. The corresponding positive control (No. 225) was moderately labelled (Fig. 1d). The anti-latex antibody (*Hev b 3*) bound, weakly, to the carbon aggregates on all filters from all four cities (example in Fig. 2b), while the latex glove used as positive control, showed strong labelling (not shown). Immunogold labelling of filters from all three seasons in Rome using antibodies to olive (Fig. 2d) and pellitory-on-the-wall, showed for both allergens a weak, positive labelling of

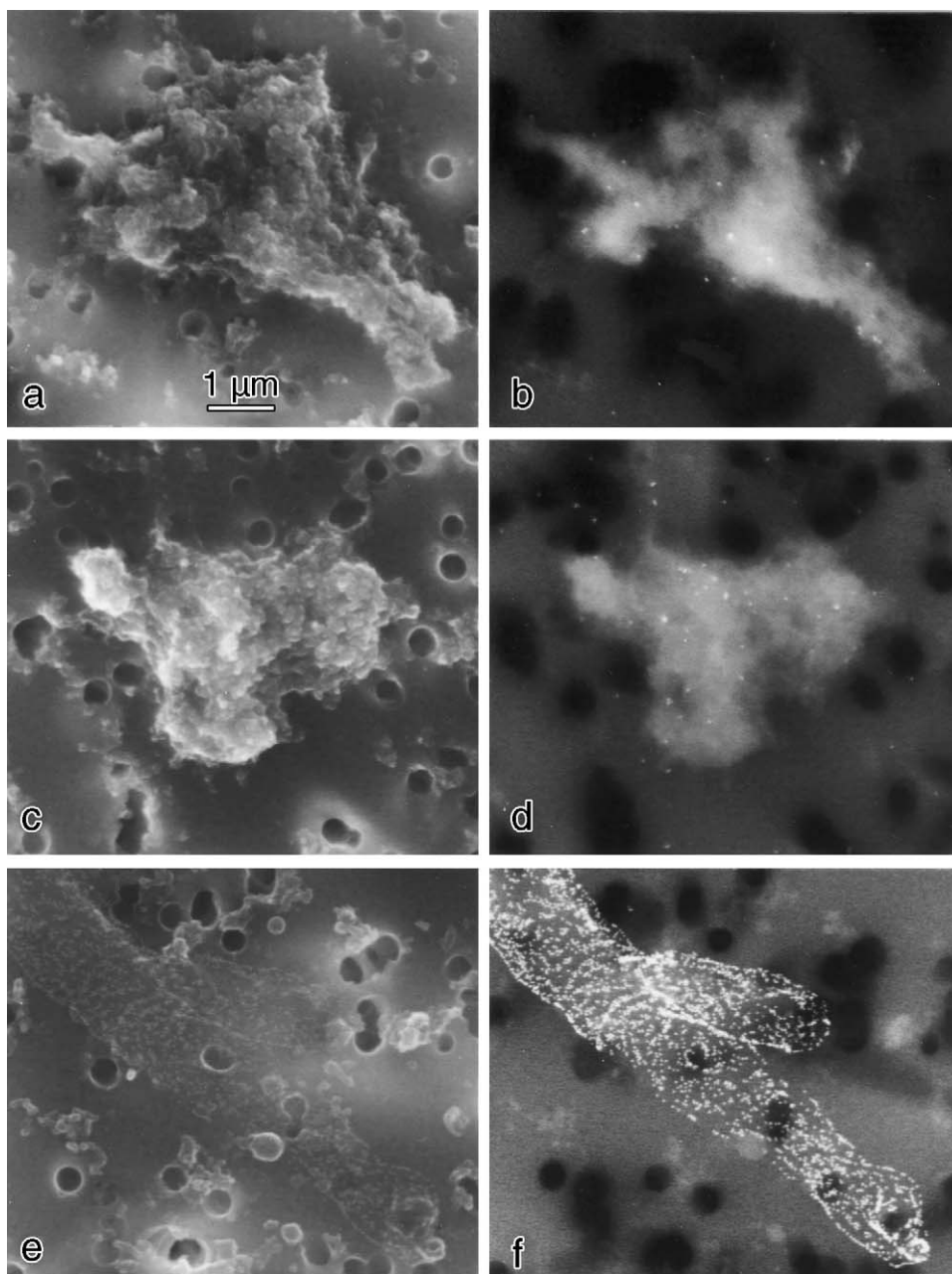


Fig. 2. Scanning electron micrographs of ambient air particulate matter (PM₁₀), collected on filters showing weak labelling of a carbon aggregate with (b) anti-*Hev b 3* against latex, (d) weak-to-moderate labelling with anti-*Ole e 1* against olive and (f) strong labelling of plant material using anti- β -glucans. The morphology of the respective aggregates are shown in (a), (c) and the plant material in (e).

carbon aggregates as well as micronic plant material. The corresponding positive controls (No. 233 and No. 224) were moderately labelled, and the antibodies were seen to bind both carbon aggregates and plant material. The polyclonal antibody to β -glucans, however, did not bind to the carbon aggregates on any of the filters from the

three most northern cities. On some of the filters from Rome, both carbon particles and plant material were labelled. The plant material, however, on all filters from all four cities were strongly labelled after incubation with the antibody to β -glucans (Table 1, Fig. 2f), as were the positive controls after incubation with MacroGuard[®]

solution. No immunogold labelling was seen on any of the negative controls (not shown in Table 1, example in Fig. 1c).

4. Discussion

Car traffic dominates the four collection sites, and previous examination of the morphological and elemental composition by transmission electron microscopy and energy-dispersive X-ray microanalysis, showed that the majority (>90%) of the ambient particles consisted of combustion particles aggregated into typical grape-like clusters (Cassee et al., 2003). Similarly, the carbon aggregates observed on all present air filters, appeared to be combustion particles originating from vehicle exhaust, as judged by morphology in SEM. Collection of ambient not sedimented particles was ensured using the PM₁₀ impactor at the low flow rate of 2 l/min. Intermitent sampling was necessary not to overload the filters for use in electron microscopy by co-deposition of particles.

Immunogold labelling of the filters showed that all antibodies tested, except birch, bound to combustion particles and aggregates of all sizes. The heavy rain (note a) in Table 1) probably “washed” down most of the particulate air pollution and caused very little material to be collected, hence no labelling was observed on the spring filter from Amsterdam. Since vehicular traffic is present at all times, the observed immunogold labelling using the anti-latex antibody, could be expected if soluble latex allergens were eluted from car tires, as indicated in previous studies (Miguel et al., 1996; Namork et al., 2004). However, since latex is known to share epitopes with allergens of vegetal origin, the possibilities of cross-reactions must be considered (Kurup et al., 1994). The observation that the antibody to timothy, bound to aggregates on filters from all seasons sampled in Oslo, also suggests a cross-reactive epitope. The immunogold labelling of the micronic particles using antibodies to pollen allergens and β -glucans, support the assumption of these particles being of vegetal origin. Glucans are polysaccharides in the cell wall of fungi and are also found in many plants (Douwes et al., 1996), which explains the very strong labelling of the plant material using anti- β -glucans. The warmer climate in Rome, and thereby more growth and higher spreading of both plant and fungal material throughout the year, may explain the weak but positive immunolabelling of the combustion aggregates with all antibodies tested on filters from all three seasons.

The finding that none of the monoclonal antibodies to the birch allergen *Bet v* 1 bound to any of the particles on the filters from Oslo and Lodz, including the

positive control allergen (No. 108), is difficult to explain but may suggest blocking of the particular epitopes on particulate-bound allergen, absence of the allergen or low affinity of the antibodies. On the other hand, the positive immunogold labelling of the plant material in the specimens and control allergen using the polyclonal antibody (Table 1), may indicate that no detectable levels of the specific allergen *Bet v* 1 were adsorbed to the combustion particles. This may be explained by the fact that the collection of ambient air particles in spring did not coincide with the peak pollen seasons for any of the northern cities that particular year (data not shown), giving low levels of the allergen at the time of sampling. Unfortunately, a limited, tight collection regime (Cassee et al., 2003) allowed only one sampling at each site and the amount of material was too small to allow further experiments. All antibodies, except the monoclonals to *Bet v* 1, bound the respective extracts used as positive controls. The control allergen extracts bound only to the combustion particles and not to the filter during incubation.

There were no marked differences between the major cities investigated with respect to density of allergen binding. The type of pollen allergen bound, however, varied according to the climate conditions. With the exception of Rome, pollen allergen levels would be expected to be low in the winter because of the colder climate. However, both latex and timothy, or cross-reactive allergens, were found to be adsorbed to the combustion particles collected in winter. With elevated mass concentrations during wintertime, due to additional heating, cold starts of cars and generally a low-lying inversion layer, traffic exposure may promote airway sensitization to some common allergens independently of season. Plant defence proteins are often allergens and may be expressed to high levels due to stress conditions from air pollution caused by heavy traffic (Cortegano et al., 2004) which may increase the allergenicity of pollens in polluted areas. Furthermore, inhalation of particulate matter may damage the airway mucosa and thereby facilitate penetration and access of inhaled allergens to the immune system. Ambient air particles are also shown to have an adjuvant effect on the allergic immune response (Nygaard et al., 2005) and particles carrying allergen must be assumed to be potent inducers of allergy. Allergen carriage by particles may thus contribute to the increased prevalence of pollen allergy in areas with high traffic pollution (Takafuji et al., 1989b).

In conclusion, immunogold labelling showed that airborne antigens from pollens, including latex and β -glucans, were adsorbed to, and hence transported by combustion particles and particles of vegetal origin in

ambient air, collected in the four European cities. Carbon aggregates have a very large surface-to-volume ratio and may function as depots of aeroallergens inhaled into the airways promoting sensitization to common allergens.

Acknowledgements

We acknowledge John Boere who was responsible for the schedule and central organization of the RAIAP sampling campaign. We thank the scientists who donated the antibodies (see Section 2). We also thank Jan Zahlin and Astri Grestad for invaluable technical assistance. This work was supported by the RAIAP project (Respiratory Allergy and Inflammation due to Ambient Particles)—A European Commission Shared-Cost Research Project QLK-CT-2000-00792.

References

- Ayuso, R., Carreira, J., Lombardero, M., Duffort, O., Peris, A., Basomba, A., Polo, F., 1993. Isolation by mAb based affinity chromatography of two *Par j* 1 isoallergens. Comparison of their physicochemical, immunochemical and allergic properties. *Mol. Immunol.* 30, 1347–1354.
- Cassee, F.R., Fokkens, P.H.B., Lesemann, D.L.A.C., Bloemen, H.J.Th., Boere A.J.F., Respiratory allergy and inflammation due to ambient particles (RAIAP); collection and characterisation of particulate matter samples from 5 European sites. Report 863001001/2003. RIVM, Bilthoven.
- Cortegano, I., Civantos, E., Aceituno, E., del Moral, A., Lopez, E., Lombardero, M., del Pozo, V., Lahoz, C., 2004. Cloning and expression of a major allergen from *Cupressus arizonica* pollen, *Cup a 3*, a PR-5 protein expressed under polluted environment. *Allergy* 59, 485–490.
- D'Amato, G., 2002. Environmental urban factors (air pollution and allergens) and the rising trends in allergic respiratory diseases. *Allergy* 57, 30–33.
- Diaz-Sanchez, D., Garcia, M.P., Wang, M., Jyrala, M., Saxon, A., 1999. Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa. *J. Allergy Clin. Immunol.* 104, 1183–1188.
- Douwes, J., Doekes, G., Montijn, R., Heederik, D., Brunekreef, B., 1996. Measurement of $\beta(1-3)$ -glucans in occupational and home environments with an inhibition enzyme immunoassay. *Appl. Environ. Microbiol.* 62, 3176–3182.
- Grote, M., Valenta, R., Reichelt, R., 2003. Abortive pollen germination: a mechanism of allergen release in birch, alder, and hazel revealed by immunogold electron microscopy. *J. Allergy Clin. Immunol.* 111, 1017–1023.
- 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., 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.
- Jensen-Jarolim, E., Ganglberger, E., Leitner, A., Radauer, C., Scheiner, O., Breiteneder, H., 1999. Nonapeptides selected by phage display mimic the binding sites of monoclonal antibodies BIP1 and BIP4 on *Bet v 1*, the major birch pollen allergen. *Int. Arch. Allergy Immunol.* 118, 224–225.
- Knox, R.B., Suphioglu, C., Taylor, P., Desai, R., Watson, H.C., Peng, J.L., Bursill, L.A., 1997. Major grass pollen allergen *Lol p 1* binds to diesel exhaust particles: implications for asthma and air pollution. *Clin. Exp. Allergy* 27, 246–251.
- Kurup, V.P., Kelly, T., Elms, N., Kelly, K., Fink, J., 1994. Cross-reactivity of food allergens in latex allergy. *Allergy Proc.* 15, 211–216.
- Lombardero, M., Quirce, S., Duffort, O., Barber, D., Carpizo, J., Chamorro, M.J., Lezaun, A., Carreira, J., 1992. Monoclonal antibodies against *Olea europaea* major allergen: allergenic activity of affinity-purified allergen and depleted extract and development of a radioimmunoassay for the quantitation of the allergen. *J. Allergy Clin. Immunol.* 89, 884–894.
- Lu, L.J., Kurup, V.P., Hoffman, D.R., Kelly, K.J., Muralis, P.S., Fink, J.N., 1995. Characterization of a major latex allergen associated with hypersensitivity in spina bifida patients. *J. Immunol.* 155, 2721–2728.
- Miguel, A.G., Cass, G.R., Weiss, J., Glovsky, M.M., 1996. Latex allergens in tire dust and airborne particles. *Environ. Health Perspect.* 104, 1180–1186.
- Namork, E., Kurup, V.P., Aasvang, G.M., Johansen, B.V., 2004. Detection of latex allergens by immunoelectron microscopy in ambient air (PM₁₀) monitored in Oslo, Norway (1997–2002). *J. Environ. Health* 67, 20–26.
- Namork, E., 1991. Double labeling of antigenic sites on cell surfaces imaged with backscattered electrons. In: Hayat, M.A. (Ed.), *Colloidal Gold: Principles, Methods and Applications*, vol. III. Academic Press, Inc., San Diego, pp. 187–208.
- Nygaard, U.C., Alberg, T., Bleumink, R., Aase, A., Dybing, E., Pieters, R., Lovik, M., 2005. Ambient air particles from four European cities increase the primary cellular response to allergen in the draining lymph node. *Toxicology* 207, 241–254.
- Ormstad, H., Gaarder, P.I., Johansen, B.V., 1997. Quantification and characterisation of suspended particulate matter in indoor air. *Sci. Total Environ.* 193, 185–196.
- Ormstad, H., Namork, E., Gaarder, P.I., Johansen, B.V., 1995. Scanning electron microscopy of immunogold labeled cat allergens (*Fel d 1*) on the surface of airborne house dust particles. *J. Immunol. Meth.* 187, 245–251.
- Petersen, A., Schramm, G., Bufer, A., Schlaak, M., Bcker, W.-M., 1995. Structural investigations of the major allergen *Phl p 1* on the complementary DNA and protein level. *J. Allergy Clin. Immunol.* 95, 987–994.
- Polosa, R., 2001. The interaction between particulate air pollution and allergens in enhancing allergic and airway responses. *Curr. Allergy Asthma Resp.* 1, 102–107.
- Polosa, R., Salvi, S., Di Maria, G.U., 2002. Allergic susceptibility associated with diesel exhaust particle exposure: clear as mud. *Arch. Environ. Health* 188, 188–193.
- Rylander, R., Lin, R.H., 2000. (1,3)-beta-D-glucan—relationship to indoor air-related symptoms, allergy and asthma. *Toxicology* 152, 47–52.
- Soligo, D., Lambertenghi-Delilieri, G., Nava, M.T., Polli, N., Catoretto, G.a.P.E.E., 1985. Scanning electron microscopy and hairy cell leukemia. *Acta Haemat.* 74, 200–204.
- RAIAP (Respiratory Allergy and Inflammation due to Ambient Particles—a European-wide Assessment), Final Report QLRT:2000-00792/2004.
- Takafuji, S., Suzuki, S., Koizumi, K., Tadokoro, K., Ohashi, H., Muranaka, M., Miyamoto, T., 1989a. Enhancing effect of sus-

- pended particulate matter on the IgE antibody production in mice. *Int. Arch. Allergy Appl. Immunol.* 90, 1–7.
- Takafuji, S., Suzuki, S., Muranaka, M., Miyamoto, T., 1989b. Influence of environmental factors on IgE production, Ciba. Found. Symp. 147, 188–201.
- Taylor, P.E., Flagan, R.C., Miguel, A.G., Valenta, R., Glovsky, M.M., 2004. Birch pollen rupture and the release of aerosols of respirable allergens. *Clin. Exp. Allergy* 34, 1591–1596.
- Wan, G.H., Li, C.S., Guo, S.P., Rylander, R., Lin, R.H., 1999. An airborne mold-derived product, beta-1,3-D-glucan, potentiates airway allergic responses. *Eur. J. Immunol.* 29, 2491–2497.
- Wiche, R., Gubesch, M., König, H., Fötisch, Hoffmann, A., Wangorsch, A., Scheurer, S., Vieths, S., 2005. Molecular basis of pollen-related food allergy: identification of a second cross-reactive IgE epitope on *Pru av 1*, the major cherry allergen. *Biochem. J.* 385, 319–327.