

Suspended particulate matter in indoor air: adjuvants and allergen carriers

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Abstract

The overall purpose of this study was to investigate how airborne house dust particles may contribute to an allergic immune response, and thereby also to asthma and other respiratory symptoms. The following aims were set: first, to quantify and characterize indoor suspended particulate matter (SPM) with regard to amount, as well as elemental and size distribution, second, to identify possible mechanisms by which SPM may affect the allergic immune response. A vast majority of the particles in SPM samples from homes in Oslo were found to be less than 2.5 μm in diameter. This $\text{PM}_{2.5}$ fraction contained, in addition to a large amount of sulfur aerosols and silicates, a lot of soot particles. Most of these were less than 1 μm in diameter. Using an immunogold labeling technique, we found that these soot particles carried cat, dog and birch allergens on their surface. These results show that indoor SPM contains a lot of potential allergen carriers, i.e. soot particles (carbon aggregates), most of them less than 1 μm in diameter and therefore able to transport allergens deep into the respiratory tree. We further found that diesel exhaust particles (DEP), which is likely the main soot component of SPM, adsorbed several well-known allergens *in vitro*. Furthermore, SPM was found to elicit a local lymph node inflammatory response, and to have an adjuvant activity on the production of IgE antibodies to ovalbumin (OA). © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: House dust; Allergens; Adjuvant; Particles; Indoor; SPM

1. Introduction

1.1. Asthma: prevalence and causes

Most investigators today agree that the prevalence of asthma has increased, as many studies from all over the world have indicated so (re-

viewed in Bates, 1995; FitzGerhald and Macklem, 1995; Mushinski, 1997). In USA, the number of people with asthma increased by 42% during the last decade, according to a report from the Centers of Disease Control (Friebele, 1996). An increase in morbidity as well as mortality from asthma during the last decades has been reported by many investigators (reviewed in FitzGerhald and Macklem, 1995; Mushinski, 1997; Meza and Gershwin, 1997). However, differences in prevalence are difficult to evaluate because of the difficulty of defining and diagnosing asthma.

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The cause for the apparently increased prevalence of asthma is unclear. However, since it is unlikely that the genetic constitution of the population investigated has undergone large changes during the last decades, the increase is most likely to be attributed to environmental factors (Leikauf et al., 1995; Sears, 1997). Some of the factors which have been discussed, are changes in building insulation, reduction in the turnover of indoor air, increased exposure to indoor pollutants (especially environmental cigarette smoke), and exposure to potent allergens (Boushey and Fahy, 1995; Newman-Taylor, 1995). It has been suggested that if changes in air pollution are contributing, the pollutants encountered indoors are likely to be more relevant than those encountered outdoors (Newman-Taylor, 1995). However, it is unlikely that any single factor will be found to be responsible. Rather, several factors act together to cause the increase in asthma and allergy that is observed.

1.2. Environmental pollution

For centuries, air pollution has been known to cause adverse health effects. An important polluting process has been the burning of coal, which was a feature of the industrial revolution (Ayres, 1997). Since World War I, there has been several episodes of excess deaths caused by air pollution (reviewed in the Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society, 1996). In many Western countries, clean air policies have been very successful and the emission of black smoke and SO₂ has diminished progressively (Ayres, 1997). However, a different type of air pollution has instead become an increasing problem, namely emissions from heavy goods vehicles, especially diesel exhaust (Ayres, 1997). It has been estimated that 38% of the particles in the ambient air over UK are diesel exhaust particles (DEP) (Quality of Urban Air Review Group, 1993).

Human exposure to air pollutants is determined by concentrations both outdoors and indoors. As it has been estimated that Western populations today spend 95% of their time indoors (Platts-Mills, 1995), indoor air pollution should be of

particular interest. Most indoor living and working environments have several air pollutant sources. Even people and pets are sources of fibers, particles and microbiologic material (Spengler and Samet, 1991). Additional pollution comes from heating and cooking combustion sources, emissions from tobacco, abrasion of surfaces, outgassing of vapors, intrusion of soil gasses, and other biologic sources (Spengler and Samet, 1991). Moreover, outdoor air pollution is brought into the indoor environment by natural and mechanical ventilation. Thus, as stated by Spengler and Samet (1991): "It should not be surprising to find indoor environments that are substantially more polluted than the nearby outdoor air".

1.3. Airborne particulates

Particles can be defined as any small bits of material or droplets either organic or non-organic, viable or non-viable, that can become airborne (Salviaggio, 1994). They can range from molecules less than 0.001 µm to pollens and spores with size between 2 and 50 µm, including also large, visible dust particles in the range of 1000 µm (Salviaggio, 1994). Particulate air pollution refers to a mixture of solid and liquid particles suspended in the air. The constituent particles typically vary in size, composition and origin (Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society, 1996). Seaton et al. (1995) calculated that, at the levels of particulate pollution recently recorded in Birmingham (100 000 particles/ml air), "each lung acinus could receive some 30 million particles and each alveolus about 1500 particles every 24 h, about 50% of which would be deposited". This illustrates the potential importance of particulate matter in relation to respiratory health.

A variety of devices are currently available for the direct measurement of particulate matter in the respirable size range, many of which collect particles on filters and typically provide results as milligrams or micrograms per cubic meter of air sampled (McCarthy et al., 1991).

The chemical composition of urban air particulate matter varies considerably. The coarse frac-

tion is reported to contain mainly mechanically generated particles, like dust from soil and other-crustal materials (Takafuji et al., 1989; Dockery et al., 1993). The fine fraction (particles less than 2.5 μm in diameter), however, is very complex (Bates, 1996; Seaton et al., 1995). This fraction is reported to consist of about half carbon and half salts, mainly ammonium sulfate and ammonium nitrate, which are mostly derived from combustion processes, especially from diesel vehicles (Seaton et al., 1995). Others have reported the fine fraction to contain a mixture of particles resulting from different combustion processes, e.g., exhaust particles (soot) and secondary particles generated by chemical reactions in the atmosphere (acid condensates, sulfates and nitrates) (Dockery et al., 1993).

Health risk has been associated mainly with particulate matter of 10 μm in diameter or less in ambient air (Choudhury et al., 1997). Particles less than 10 μm in diameter are often referred to as “inhalable”. The terms PM_{10} (particulate matter < 10 μm in mean aerodynamic diameter) or suspended particulate matter (SPM) are often used for this particle fraction. PM_{10} express the mass concentration rather than the number of particles, and is measured in terms of $\mu\text{g}/\text{m}^3$. This fraction is divided into a coarse fraction (> 2.5 μm in diameter) and a fine fraction (< 2.5 μm in diameter) (Clayton et al., 1993), which is also often referred to as $\text{PM}_{2.5}$. The term aerodynamic diameter, which is often used when referring to particle size, is defined as the diameter of a spherical particle having unit density that has the same settling velocity as the particle in question (Schlesinger, 1985).

1.4. Particulate matter and respiratory symptoms

Although dramatic air pollution episodes associated with excess mortality are now rare in most of the world, several pollutants may be responsible for adverse health effects at current levels of exposure. The increase in allergic diseases (including asthma) over recent decades has been linked to an increase in environmental pollutants (Björkstén, 1997; Miyamoto, 1997; Schäfer and Ring, 1997). Common air pollutants, almost without

exception, present a challenge to asthmatics. Most of the epidemiological data relate worsening of asthma to changes in pollutant levels (Bates, 1995). The major air pollutants linked to asthma are sulfur dioxide (SO_2 , acid aerosols), ozone (O_3), nitrogen dioxide (NO_2) and particulate matter (Devalia et al., 1996). Several papers have reported effects of these pollutants on lung function and respiratory symptoms, and especially particulate matter has often been found to cause respiratory symptoms (reviewed in Pope et al., 1995a). In a recent study, Ackermann-Liebrich et al. (1997) investigated health effects from long term exposure to air pollutants, and found significant and consistent effects on functional vital capacity (FVC) and forced expiratory volume per 1 s (FEV_1) for NO_2 , SO_2 and PM_{10} in the total population studied. PM_{10} showed the most consistent effect, and gave a 3.4% reduction in FVC per 10 $\mu\text{g}/\text{m}^3$.

Recently, a cohort of over half a million US adults was studied, and significant associations were found between lifetime exposure to sulfate aerosols and fine particles and some causes of mortality (Pope et al., 1995b). Several other studies have also demonstrated that particulate matter in ambient air has adverse effects on respiratory health (reviewed in Pope et al., 1995a; Gordian et al., 1996; Choudhury et al., 1997; Lipsett et al., 1997; Peters et al., 1997; Hajat et al., 1999; Goren et al., 1999). In spite of many observations of a clear association between particulate matter and respiratory symptoms, the mechanisms of the adverse effects are still not fully understood.

1.5. Aeroallergens

Sensitization to allergens usually occurs across the mucosal surfaces of the gastrointestinal or respiratory tract or through the skin. In respiratory allergy, in which the airways constitute the prime site for allergic reactions, the ability of an allergen to become airborne is of particular importance. Aeroallergens are airborne allergens which are usually constituents of airborne particles, either the original source (mite feces, pollen grains etc.) or other types of particles (dust). A variety of aeroallergens are important in asthma,

including indoor allergens from house dust mites, cats, dogs, cockroaches, and outdoor allergens from pollen (Utell and Looney, 1995; Platts-Mills, 1997a). Many asthmatics have been found to be sensitized to common allergens of indoor and outdoor origin (Boulet et al., 1997). Indoor allergens are probably of particularly great importance, as most populations in the Western world probably spend 95% or more of their time indoors in tightly sealed, overheated buildings (Platts-Mills, 1995). A study by Gelber et al. (1993), showed that in a cohort of 114 asthmatics and 114 control subjects, 38% of the asthmatics and only 8% of the control subjects were allergic to one of the three allergens: mite, cat and cockroach. Platts-Mills (1997b) concludes that children who become allergic to foreign proteins in their homes, have an increased risk for asthma and that continued exposure contributes to morbidity.

The relative importance of the individual allergens varies around the world, depending on climatic conditions and living habits. For example in UK, the house dust mite is considered to be a very important allergen, as 80% of asthmatic children are sensitized to mites (Mygind et al., 1996). In Scandinavia, however, mite allergens are not assumed to be of such great importance (Munir, 1994). In Sweden, both the prevalence and levels of cat (*Fel d 1*) and dog (*Can f 1*) allergens in the dust have been found higher than those of mite allergens (Munir, 1994). Moreover, asthmatic children in Sweden are usually sensitized to furred pets and pollen (Croner and Kjellman, 1992).

1.6. Possible effects of SPM on the allergic immune response

It is still not fully known in what way and to what extent airborne dust particles may contribute to asthma and respiratory symptoms, although some possibilities have been studied and discussed.

SPM may contain components causing a non-specific irritation in the airways. This may contribute to bronchial hyper-responsiveness, making the airways more sensitive to other environmental components, including allergens. For example, two recent studies showed particulate matter to

have free radical activity (Gilmour et al., 1996; Li et al., 1996). Li and coworkers also demonstrated particulate matter to cause inflammation and epithelial injury in rat lungs. This suggests that particulate matter may generate both non-specific and allergen-specific hyper-reactivity by provoking airway inflammation. This mechanism has also been suggested by Wardlaw in a review on air pollutants (Wardlaw, 1993).

In the same review, Wardlaw hypothesized that particulate matter may, in some way, modify the immune response. Thus a second possibility is that SPM may modify the immune response by promoting IgE production. SPM has actually been shown to cause enhanced production of specific IgE to ovalbumin (OA) (Takafuji et al., 1989). Moreover, DEP, which is probably an important component of SPM, has also been found to have such an adjuvant effect (Takafuji et al., 1987; Suzuki et al., 1993; Fujimaki et al., 1994, 1997; Løvik et al., 1997). This means that SPM, either in its entirety or only as individual particle types, may increase the production of specific IgE to environmental allergens.

The possibility has also been considered that airborne particles may carry allergens in ambient air (Behrendt et al., 1992), as the presence of allergenic activity has been demonstrated in various size fractions of airborne particles (Luczynska et al., 1990; Spieksma et al., 1990; Rantio-Lehtimäki et al., 1994; Schäppi et al., 1996). This may be an important way of transporting allergens into the lower respiratory tract.

The overall aim of the study reported here was to investigate how airborne house dust particles may contribute to an allergic immune response, and thereby also to asthma and other respiratory symptoms. To do this, quantification and characterization of indoor SPM was first carried out with regard to amount, as well as elemental and size distribution. With regard to mechanisms by which SPM may affect the allergic immune response, the following questions were asked: can well-known allergens like *Fel d 1* (cat), *Can f 1* (dog), *Bet v 1* (birch pollen) and *Der p 1* (mite) be detected on the surface of identifiable airborne house dust particles by immunoelectron microscopy? Furthermore, can allergens be attached

in vitro to DEP, which is possibly an important constituent of the SPM soot fraction? And finally, do airborne house dust particles in a mouse model have an adjuvant activity on the response to OA, with regard to a local cellular response and/or the production of specific IgE?

2. Materials and methods

2.1. Quantification and characterization of SPM in indoor air

SPM was collected on polycarbonate filters from 29 households in the city of Oslo from early March 1994 through January 1995 using a SKC 224-PCXR7 pump (SKC, Inc., USA). The pump was used at a steady flow of 1 air/min through the filter. The sampling proceeded for 24 h. Under the sampling conditions used, which are described in more detail in the original report (Ormstad et al., 1995), the dust particles collected were mainly less than 10 μm in diameter, i.e., PM_{10} or SPM. Two parallel samples were taken in each household, both in the living room. In addition to the two indoor samples, one outdoor sample was taken for comparison. The samples were all weighed, and a transmission electron microscope (TEM) analysis was carried out on the indoor samples from 12 households collected consecutively in the period from 15th September to 13th December 1994. The specimens were examined in a JEM-100CX TEM operated at 80 keV, which was coupled to an energy dispersive X-ray spectrometer (Link ISIS system) for performance of microanalysis of the particles. The analysis procedure is described in more detail elsewhere (Ormstad et al., 1995). Statistical analysis was performed using the Minitab[®] software for PC.

2.2. Immunogold labeling and scanning electron microscopy of allergens on the surface of collected airborne house dust particles and allergen-exposed DEP

A Dymax 30 vacuum pump (Austen Pumps Ltd., UK) with a flow of 4 l/min was used to collect airborne particles for 24 h in different homes. The low flow was used to make sure that the dust

collected was truly airborne. The particles collected during this sampling, which were all essentially less than 10 μm , will be referred to as SPM particles.

To study the presence of the cat allergen Fel d 1, SPM was collected on polycarbonate filters from 10 homes, 5 with and 5 without a cat. The presence of Can f 1 (dog allergen) was investigated in 5 homes with, and 5 homes without a dog. Bet v 1 (birch pollen allergen) was studied in 5 samples collected in April/May and 5 samples collected in the autumn/winter period. Finally, the house dust mite allergen Der p 1 was investigated in 10 randomly selected homes.

The DEP (Standard Reference Material; SRM 1650) used in this study were obtained from US National Institute of Standards and Technology (NIST), Gaithersburg, MD, and has been described in more detail elsewhere (Løvik et al. 1997). Analyzed in the scanning electron microscope (SEM), these particles are morphologically indistinguishable from the soot particles regularly found in SPM samples. The procedure for binding of allergens to DEP exposed polycarbonate filters has been described in more detail in the original publication (Ormstad et al., 1998a).

Preparations from the SPM or DEP exposed filters were subjected to immunogold labeling using monoclonal antibodies against the respective allergens as the primary antibody and a goat anti-mouse IgG conjugated with 30 nm gold particles as the secondary antibody. As negative controls, monoclonal antibodies of the same isotype, but with irrelevant specificities were used. All the monoclonal antibodies were isotype IgG1 and they were used at 5 or 10 $\mu\text{g}/\text{ml}$. The immunogold labeled specimens were examined in a SEM, in which two different “modes” (depending on type of electrons detected) were used; backscatter electron imaging to visualize the gold particles, and secondary electron imaging to study the morphology of the gold labeled SPM particles.

2.3. The popliteal lymph node assay in mice: a method for studying the adjuvant activity of SPM on specific IgE production and on the local lymph node response

Groups of female Balb/c mice (6–8-week old)

were injected in the right hind footpad with one of the following preparations: SPM + OA, DEP + OA, SPM alone, or OA alone on Day 0. On Day 20, 8 mice from each group were exsanguinated, and the popliteal lymph node (PLN) was taken out. The PLN weight, cell numbers and cell proliferation were determined, as well as serum IgE against OA. The lymph node parameters were measured using the following methods, respectively: counting of cells in a Coulter counter, ^3H -labeled thymidin incorporation in PLN cell suspensions cultured without in vitro antigen stimulation on microtiterplates, and ELISA. On Day 21 all remaining mice were reinjected with OA alone, except for eight SPM + OA mice which were given medium, as controls. On Day 26 and Day 33 another 8 mice from each group were exsanguinated, and the PLN was taken out. The same parameters were measured as on Day 20.

As a measure for the local lymph node response, we calculated the indexes of the PLN weight, cell numbers and cell proliferation. The index is the value of the right PLN (inoculated) divided by the value of the left PLN (normal). The data were analyzed with the SigmaStat[®] Statistical Analysis System for the PC Version 1.0 (Jandel Scientific, Erkrath, Germany).

3. Results

3.1. SPM in Norwegian homes

The concentrations of SPM measured in 29 homes were found to vary from 9 to 56 $\mu\text{g}/\text{m}^3$ (median value 26 $\mu\text{g}/\text{m}^3$) (Ormstad et al., 1997). In 25 of the 29 homes, a higher indoor than outdoor SPM concentration was measured, and the median indoor/outdoor ratio was found to be 1.43. Furthermore, a significant correlation was found between the indoor and outdoor SPM concentrations. In homes with electrical convection ovens, the average SPM concentrations were significantly higher than in homes with radiators.

Analysis in TEM showed that silicates and carbon aggregates (soot) were the particle types most frequently found in the SPM samples. Moreover, “pure” particles of sulfur, iron, and calcium

were all frequently present in the samples. In addition, different amounts of particles of organic origin (fibers or pleomorphic structures) and various types of complex inorganic particles were found. Sodium and sulfur; calcium and sulfur; sodium and chlorine; and potassium and chlorine were typical combinations observed. Morphologically, these particles were usually indistinguishable from each other and from the silicates.

Concerning size distribution, the vast majority of particles were less than 2.5 μm in diameter. This $\text{PM}_{2.5}$ fraction contained a substantial amount of soot and sulfur particles, usually less than 1 μm in diameter. Also many silicates were found to be smaller than 2.5 μm in diameter. The coarse fraction (particles > 2.5 μm in diameter) was found to consist mainly of organic material, silicates and larger soot aggregates. Fig. 1 shows transmission electron micrographs of some of the most frequently found particles in the SPM samples.

3.2. SPM as allergen carriers

Fel d 1 was detected on the surface of SPM particles, and was almost exclusively found attached to one particular type of SPM, namely soot particles (carbon aggregates) (Ormstad et al., 1995). Both smaller (< 1 μm) and larger (10 μm) aggregates were found to have *Fel d 1* attached to their surface (Fig. 2). No significant labeling was found on other types of SPM particles, neither organic nor non-organic. *Fel d 1* was detected in all 10 homes investigated. Subjectively estimated, however, the soot particles were found to be more densely labeled in homes with a cat.

Furthermore, we detected both *Can f 1* and *Bet v 1* allergens on the surface of SPM soot particles (Ormstad et al., 1998a), although for both allergens to a lesser extent than previously found with *Fel d 1* (Ormstad et al., 1995), subjectively estimated. *Can f 1* was detected both in homes with and without a dog. However, the labeling of *Can f 1* on the soot particles was estimated to be denser in homes with a dog. Concerning *Bet v 1*, the samples collected in April/May showed much more dense labeling of soot particles than the

samples collected during the autumn and winter period, again subjectively estimated. *Der p 1* was not detected on the surface of the SPM soot particles in any of the 10 homes investigated.

We found that all the allergens investigated bound to DEP in vitro, including *Der p 1*, although this allergen was not found naturally bound to soot particles in SPM (Ormstad et al., 1998a).

3.3. The adjuvant activity of SPM

We investigated the effect on the local lymph node response by measuring the PLN weight, cell numbers and cell proliferation. Furthermore, the adjuvant activity on the IgE production was studied by measuring the level of OA specific IgE. DEP was included in the study as a particle preparation with known adjuvant activity.

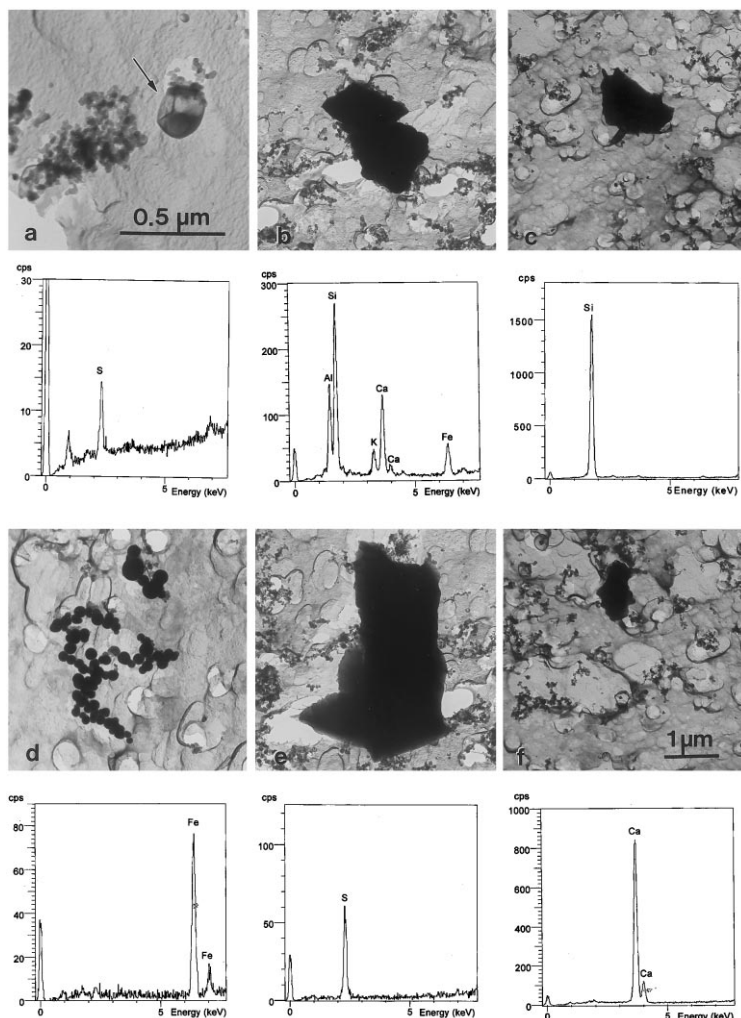


Fig. 1. Transmission electron micrographs of different types of particles frequently found in indoor SPM samples, and their corresponding XRMA spectra. (a) A carbon aggregate (soot particle) and a sulfur aerosol (arrow), both with a characteristic morphology and size; (b) a typical complex silicate regarding composition of elements; (c) a pure silicate; (d) clusters of characteristic round pure iron particles; (e) a typical sulfur particle of a type more seldom found than the one in Fig. 1a; (f) a pure calcium particle. (Reproduced from Ormstad et al., 1997, with permission).

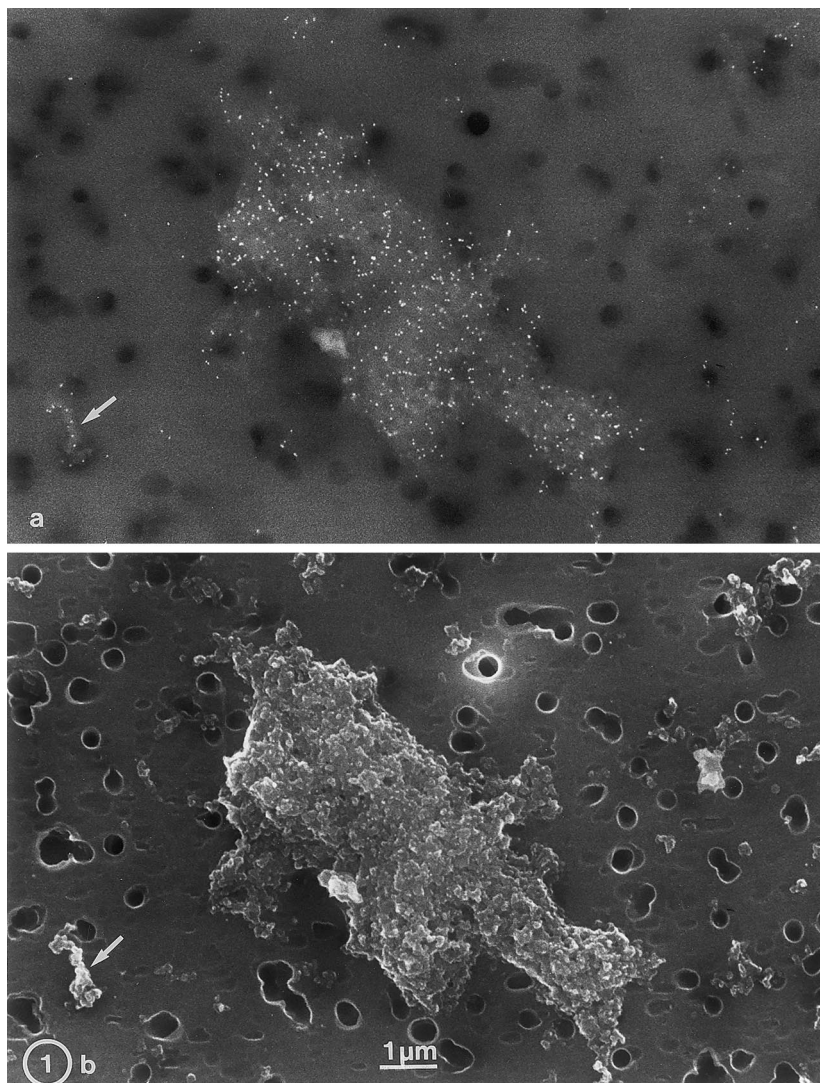


Fig. 2. Backscatter (a) and secondary (b) electron micrographs of carbon aggregates in an airborne dust sample from a household with a cat, labeled with mouse IgG1 anti-Fel d 1. The secondary antibody is goat anti-mouse IgG conjugated to 30 nm gold particles. Note the gold labeling also on the smaller aggregates ($< 1 \mu\text{m}$) (arrows). $\times 13\,000$. (Reproduced from Ormstad et al., 1995, with permission).

Both DEP + OA, SPM + OA, and SPM alone gave significantly increased weight ($P < 0.05$), cell number and cell proliferation indexes as compared to OA alone (Ormstad et al., 1998b). Thus SPM injected together with OA, as well as SPM alone gave an augmented PLN response as compared to OA alone. This means that SPM was found to elicit a local lymph node response, both when injected alone and together with OA. DEP

was also found to have adjuvant activity when injected together with OA, but has previously been found (Løvik et al., 1997) to cause no lymph node response when injected alone.

Furthermore, the groups of mice immunized with SPM + OA or DEP + OA both showed a significantly increased production of OA-specific IgE on Day 26, five days after reinjection with OA, as compared to the mice immunized with OA

alone ($P < 0.05$) (Fig. 3). Enhanced IgE production was not found when SPM was given alone in the first injection. The SPM + OA control group reinjected with medium on Day 21, showed no clear increase of IgE levels. These results show that SPM enhances the production of OA-specific IgE, when injected together with OA, compared to OA injected alone.

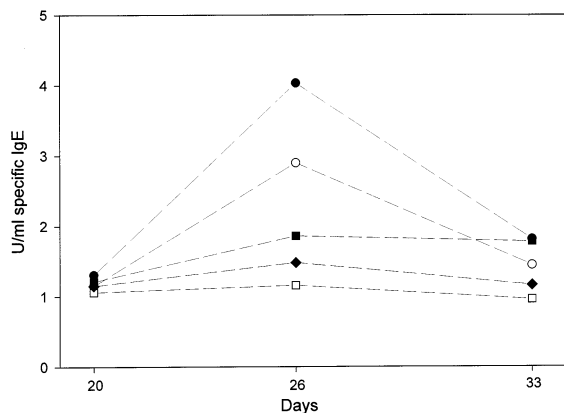


Fig. 3. OA-specific IgE values for mice immunized with 50 µg SPM + 50 µg OA (○), 50 µg DEP + 50 µg OA (●), 50 µg OA (■) or 50 µg SPM (□) on Day 0, and reinjected with 50 µg OA on Day 21. One group was injected with 50 µg SPM + 50 µg OA, followed by medium on Day 21 (◆). Median values (arbitrary units) for groups of eight mice are given. (Reproduced from Ormstad et al., 1998a,b with permission).

4. Discussion

4.1. SPM in Norwegian homes

The vast majority of the SPM was found to be particles less than 2.5 µm in diameter, i.e., belonging to the fine fraction (or PM_{2.5} fraction). These smaller particles are probably more liable to affect respiratory health, as they are able to penetrate deep into the respiratory tree. Özkaynak and Thurston (1987) reported increased mortality associated with the fine fraction rather than with the total amount of airborne particles.

Silicates, soot particles (carbon aggregates) and sulfur aerosols were the three particle types most frequently found in the SPM samples.

Silicates may have adverse respiratory health effects, as silica (silicon oxide) has previously been found to affect respiratory health (Reiser and Last, 1979; Okada et al., 1993). About 90% of the silicates found in SPM were of complex nature, as they frequently contained elements like aluminum, calcium, potassium and iron, which may possibly also contribute to the adverse effects of SPM (Dusseldorp et al., 1995). Aluminum, calcium, potassium and iron were also often identified in other complex (or pure) particles. The bulk of the silicates were between 2 and 4 µm in diameter, which means that they were found both in the fine fraction (<2.5 µm) as well as in the coarse fraction (>2.5 µm).

Soot particles (carbon aggregates) were always identified in the SPM samples, and often to a large extent. It is likely that a considerable amount of these soot particles originates from diesel exhaust. Imaged in SEM, the soot particles and commercially available DEP are morphologically indistinguishable. Further, it is known that diesel engines release much more particles per mile than petrol engines (Vostal, 1980; Frew and Salvi, 1997). Moreover, it has been proposed that the largest source of airborne particles arising from man made activities in urban areas is diesel engines (BéruBé et al., 1997). However, it cannot be excluded that other combustion processes may also contribute to the soot found in SPM.

DEP has been found to have an adjuvant effect on the production of specific IgE to certain antigens (Takafuji et al., 1987; Suzuki et al., 1993; Fujimaki et al., 1994, 1997; Løvik et al., 1997). Moreover, studies performed by Devalia et al., (1996) indicated that DEP may adversely affect the mucociliary barrier of the airways. It is alarming that these health-affecting SPM particles were mainly found to be smaller than 1 µm in diameter, which means that they can penetrate deep into the respiratory tree.

The third frequently found particle type was sulfur aerosols, which were also generally less than 1 µm in diameter. A high proportion of sulfur in the fine fraction has also been reported by other authors (Clayton et al., 1993). Sulfuric acid aerosols are known to result from oxidation of SO₂ in combination with humidity in the air,

and they have a typical diameter of 0.3–0.6 μm (Norwegian State Pollution Control Authority, 1992). Concerning the possible adverse effect from these sulfur aerosols, it has been reported by several authors that sulfuric acid aerosols give increased respiratory symptoms (Koenig et al., 1989; Spektor et al., 1989). Besides, it has been reported in humans that 77% of inhaled aerosols of sulfuric acid will be retained in the alveolar region of the respiratory tract (Amdur et al., 1952).

In summary, these results indicate that SPM sampled in Norwegian homes contains several different particles, which are liable to cause respiratory symptoms.

4.2. SPM as allergen carriers

The immunogold labeling technique used, revealed that important allergens were attached to the surface of particles in SPM samples, that is, *Fel d 1* (cat), *Can f 1* (dog) and *Bet v 1* (birch pollen) (Ormstad et al., 1995, 1998a). In contrast, the mite allergen *Der p 1* was not detected in any significant amount in SPM samples.

Previously, the presence of allergenic activity has been demonstrated in various size fractions of airborne particles, with ELISA (Luczynska et al., 1990; Rantio-Lehtimäki et al., 1994; Schäppi et al., 1996) as well as RAST inhibition assay (Spieksma et al., 1990). Importantly, it has not been studied previously whether the allergens are associated with particular types of airborne particles. The methodology described and used in our studies, made it possible to conclude that *Fel d 1*, *Can f 1* and *Bet v 1* allergens were attached almost exclusively to the soot particles of SPM. Both *Fel d 1* and *Can f 1* were found in homes both with and without the respective pet. However, specimens from homes with a pet, showed more densely labeled soot particles, subjectively estimated. Other studies have revealed the presence of these allergens in settled dust from homes without the respective pet (Wood et al., 1988; Schou et al., 1991; Munir et al., 1993, 1994; Sakaguchi et al., 1993; Quirce et al., 1995). Concerning *Bet v 1*, samples collected in the autumn and winter period showed only weak gold labeling

compared to the samples from April/May. This may not be very surprising, as April/May is the most intense period for birch pollen in Norway. Also, others have reported the finding of low quantities of pollen allergens in airborne samples outside the pollen season (Spieksma et al., 1995).

Since DEP possibly contribute considerably to the soot fraction of SPM, we studied the ability of DEP to bind the same allergens in vitro (Ormstad et al., 1998a). This study showed that DEP bound *Fel d 1*, *Can f 1* and *Bet v 1* both from partially purified extracts and crude extracts. Also *Der p 1*, which was not detected on SPM, was found to bind to DEP, when a crude extract was used. DEP has also been reported by other authors to bind proteins (allergens) (Muranaka et al., 1986; Knox et al., 1997).

Whether a protein binds to DEP/soot or not, may depend on the charge of the protein (discussed in Ormstad et al., 1998a). All the allergens included in the DEP study have isoelectric points lower than the pH used (pH 7.4) (Chapman and Platts-Mills, 1980; Ipsen and Løwenstein, 1983; Didierlaurent et al., 1984; Leitherman and Ohman, 1984; Ford and Kemeny, 1992), thus they were all negatively charged. It has been suggested that negative charges may promote the binding to DEP, as it was found that a negatively charged allergen bound to DEP, whereas a positively charged allergen did not (Knox et al., 1997). The actual binding of allergens to soot/DEP in ambient air, however, must in addition depend on the opportunity of the allergen to meet the particles under appropriate physical circumstances, e.g., in an airborne form. This might be an explanation why *Der p 1* was not found on SPM. The *Der p 1* allergen, which is easily water soluble, is associated with the membrane enveloped mite faecal particles, which are typically 10–40 μm in diameter (Tovey et al., 1981a). Perhaps, the indoor environment does not provide the appropriate conditions for *Der p 1* to be liberated and dissolved. Also, others have reported the absence of *Der p 1* from airborne particle samples, where *Fel d 1* and *Can f 1* allergens are present (Custovic et al., 1996). Moreover, Tovey et al. (1981b) found no airborne *Der p 1* in undisturbed rooms. However, during disturbance, *Der p 1* was detectable

in some homes, but the allergen was mainly associated with particles larger than 10 μm .

In contrast to *Der p 1*, *Fel d 1* and *Can f 1* are more liable to get airborne, both being present in the saliva of the cat and dog, respectively (reviewed in Schou, 1993; Bessot et al., 1994). It is therefore likely that these allergens may become airborne as droplets of saliva, and then attach to the “sticky” soot particles in the environment. Other authors have also reported the finding of *Fel d 1* (Luczynska et al., 1990; de Blay et al., 1991) and *Can f 1* (Custovic et al., 1997) associated with airborne particles as small as $<2.5 \mu\text{m}$. *Bet v 1* is known to be released in abundance from the birch pollen grains under humid conditions (Grote et al., 1993; Vrtala et al., 1993; Ormstad, unpublished results). Thus *Bet v 1* may become airborne and stick to the soot particles in ambient air under humid conditions. Spielsma et al. (1995) found grass pollen allergenic activity in the aerosol fraction containing particles $<0.6 \mu\text{m}$, which showed that airborne pollen allergen activity was associated with particles much smaller than intact pollen grains. Similar findings were achieved by Sch  ppi et al. (1996), who demonstrated *Bet v 1* in all particle size ranges studied, including the smallest fractions ($<1 \mu\text{m}$).

The major issue concerning aeroallergens is whether there is a causal relationship between allergen exposure and asthma. A new allergy textbook like “Essential Allergy” from 1996, states, in fact, that there is now convincing evidence that exposure to allergens is the primary cause of the development of asthma in children and young adults (Mygind et al., 1996). Several authors have observed higher counts of mites in the homes of asthmatics compared to homes of control subjects (Korsgaard, 1983; Bj  rnsson et al., 1995). Others, however, have reported to find no such correlation when measuring mite allergen levels (Marks et al., 1995a). Moreover, it has been reported that a large reduction in house dust mite allergen concentration, particular in the bed, resulted in only a modest reduction in airway hyper-responsiveness and improvement in symptoms in mite allergic subjects with asthma (Marks et al., 1995b). Also for dog and cat allergens, there is no clear evidence of higher allergen concentrations in

the homes of asthmatics (Chan-Yeung et al., 1995; Ingram et al., 1995). Thus, there are still studies contradicting a clear relationship between indoor allergen exposure and asthma. Platts-Mills (1995) discusses some factors that may obscure the relationship between measurements of allergen in houses and symptoms of asthma, and perhaps the most important one is that the quantification of cat, dog and mite allergen in carpets and beds (which is mostly done in the “relationship” studies), do not reveal the amount of allergens entering the lung. Thus, it is probably misleading to conclude, on the basis of the lack of demonstration of a correspondence between measured allergens in settled dust and asthmatic symptoms, that there is no causal relationship between allergen exposure and asthma. Measurements of airborne allergens associated with different fractions of SPM would be more relevant for the estimation of exposure.

4.3. The adjuvant activity of SPM

In the study described, SPM was found to have an enhancing effect on the PLN response, not only when injected together with OA, but also when injected alone, compared to OA alone (Ormstad et al., 1998b). This indicates that SPM may cause significant inflammatory response in the draining lymph node. L  vik et al. (1997) did not find a similar effect with DEP alone. That is, DEP was only found to cause an augmented PLN response when inoculated together with an antigen (OA). The fact that SPM alone, in contrast to DEP alone, caused an augmented PLN response may not be very surprising. As already described, soot particles in SPM were found to carry important allergens, thus SPM may, in fact, be regarded as a mixture of DEP (soot) and allergens. In addition, SPM contains a lot of different types of particles as found in the TEM analysis, and most likely also organic substances like endotoxin, which cannot be excluded as contributors to an enhanced local lymph node response.

When injected together with OA, SPM was found to cause an increased production of specific IgE to OA, after reinjection with OA. This means that SPM had an adjuvant activity on the produc-

tion of specific IgE to OA. SPM has also previously been found to have an adjuvant effect on the production of IgE to OA, using intranasal immunization (Takafuji et al., 1989). A similar adjuvant effect of DEP has also been found in several studies (Takafuji et al., 1987; Suzuki et al., 1993; Fujimaki et al., 1994, 1997; Løvik et al., 1997). Also in our study, this adjuvant effect was found with DEP, which was included in the study as a particle preparation with known adjuvant effect.

An interesting question regarding these findings is whether particular particle types in SPM, for example soot (DEP), are responsible for this adjuvant effect, or if it is due to additive, or even synergistic effects between more components. It is likely that the soot particles in SPM are at least partly responsible for the observed adjuvant effect, as DEP has been shown to have a similar effect. However, it cannot be excluded that also other SPM particles may contribute. There are several differences between SPM and DEP, which makes it difficult to compare their adjuvant activities. Thus it is not possible, from this study, to say whether the soot particles alone are responsible for the adjuvant effect of SPM.

Another question is, what is the mechanism behind the adjuvant activity of SPM and DEP on the IgE production? It has recently been suggested that DEP may affect the immune response via the modulation of cytokine production in mast cells, as it was found that DEP gave increased production of IL-4 and IL-6 from mouse marrow-derived mast cells (Saneyoshi et al., 1997). In addition, the allergen-carrying properties of soot/DEP (Ormstad et al., 1998a) may be an important factor for the adjuvant activity. If the OA proteins are still bound to the particles when they reach the immune system, OA might be released to the system slowly, giving a “depot effect”. This could also be the case in the real-life situation when depots of environmental allergens adsorbed to particles enter the airways and are slowly released, causing an adjuvant effect on IgE production. Finally, the particles may carry

adsorbed substances with known adjuvant effect, like endotoxins and β -glucans.

5. Conclusions

A vast majority of particles in SPM samples from homes in Oslo were found to be less than 2.5 μm in diameter, and thus are liable to penetrate deep into the respiratory tree. This $\text{PM}_{2.5}$ fraction contained, in addition to much sulfur aerosols and silicates, a lot of soot particles, most of them being less than 1 μm in diameter. Using an immunogold labeling technique these soot particles were found to carry common allergens on their surface. These results show that indoor SPM contains a large amount of potential allergen carriers, i.e. soot particles (carbon aggregates), most of them being less than 1 μm in diameter. We also found that DEP, which is probably a main soot component of SPM, adsorbed several well-known allergens *in vitro*.

Furthermore, SPM was found to elicit a local lymph node response after footpad injection in mice, and to have an adjuvant activity on the production of IgE antibodies to OA as a model allergen. Probably, the adjuvant activity is at least partially due to the soot particles in SPM, as the same effects are found with DEP. However, it cannot be excluded that also other SPM particles and adsorbed substances may contribute to the adjuvant effect. Finally, our results indicate that SPM may provoke a local inflammatory response.

Our findings support the notion that SPM in the environment may contribute to the allergic response and the inflammation of asthma in at least three different ways. First, SPM may have an irritating effect on the airways, resulting in an inflammatory response, which again may disrupt the epithelial barrier and thereby increase the sensibility to environmental allergens. SPM may perhaps also contribute to the chronic inflammation that is an essential component of asthma. Second, soot particles in the SPM may carry important allergens down into the airways. Finally, SPM may increase the IgE production to allergens present, by modulating the immune response.

Acknowledgements

The present work was supported by a Ph.D. grant (BA30854) from the Research Council of Norway. The author acknowledges her supervisors Professor B.V. Johansen, Dr. Per-Ivar Gaarder and Dr. Martinus Løvik, as well as Dr. Ellen Namork, Åse Eikeset, Else-Carin Groeng, Rita-Bente Leikvold and Berit Stensby for expert help and technical assistance. The author is also indebted to Dr. Audun Aase for valuable help and discussions.

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