Dose and Time Dependency of Inflammatory Responses in the Mouse Lung to Urban Air Coarse, Fine, and Ultrafine Particles From Six European Cities

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We investigated the dose and time dependency of inflammatory and cytotoxic responses to size-segregated urban air particulate samples in the mouse lung. Coarse (PM_{10-2.5}), fine (PM_{2.5-0.2}), and ultrafine (PM_{0.2}) particles were collected in six European cities (Duisburg, Prague, Amsterdam, Helsinki, Barcelona, Athens) in selected seasons using a modified Harvard high-volume cascade impactor. Healthy C57Bl/6J mice were intratracheally exposed to the particulate samples in a 24-h dose-response study (1, 3, and 10 mg/kg) and in 4-, 12-, and 24-h time course studies (10 mg/kg). After the exposures, the lungs were lavaged and the bronchoalveolar lavage fluid (BALF) was assayed for indicators of inflammation and tissue damage: total cell number,
cell differential, total protein, and lactate dehydrogenase (LDH) and cytokine (tumor necrosis alpha [TNF-α], interleukin-6 [IL-6], and keratinocyte-derived chemokine [KC]) concentrations. In general, PM10-2.5 samples had higher inflammatory activity than PM0.2-0.5 samples. PM10-5 samples showed negligible inflammatory activity. PM10-2.5 and PM2.5-0.5 samples caused large increases in BALF cytokine concentrations at 4 h, but not at 12 or 24 h, after exposure. The BALF total cell number and total protein concentrations increased significantly at 12 h for both the PM10-2.5 and PM0.2-5 samples, but only PM10-2.5 samples produced consistent, significant increases at 24 h after exposure. There was more heterogeneity in BALF cytokine and neutrophil cell number responses to PM0.2-5 samples than to PM10-2.5 samples between the sampling campaigns. Thus, particle size, sources, and atmospheric transformation processes affect the inflammatory activity and response duration of urban air particulate matter in the mouse lung.

Epidemiological studies have shown significant regional heterogeneities in the exposure-response relationships of urban air thoracic particles (PM10; particle diameter (Dp) < 10 μm) with the short-term mortality (Samoli et al., 2005) and hospital admissions (Atkinson et al., 2001) in Europe. The relationships have been stronger per unit of mass concentration for cardiovascular mortality and hospital admissions in southern cities with higher photochemical pollution, and for respiratory mortality in eastern cities. Moreover, there have been stronger than average exposure-response relationships for mortality and for a whole variety of morbidity and functional outcomes among cardiorespiratory patients and children in association with combustion-derived particles from local traffic (Hoek et al., 2002; Laden et al., 2000; Lanki et al., 2006; Penttinen et al., 2006), local coal combustion (Clancy et al., 2002; Laden et al., 2000), and a poorly controlled steel mill (Pope et al., 1991). With the exception of the Utah Valley steel mill case (Ghio, 2004), there is little experimental evidence to show that an observed heterogeneity is due to differences in toxic properties of ambient air particles. The alternative reasons include, for example, climatic and population-based differences.

Urban air PM10 is a highly complex mixture of different size solid and liquid particles originating from a large variety of anthropogenic and natural sources. Epidemiological studies have most often given stronger exposure-response relationships for morbidity and mortality outcomes in association with fine particles (PM2.5; Dp < 2.5 μm) than with PM10 (WHO, 2003; U.S. EPA, 2004). However, urban air coarse thoracic particles (PM10-2.5; 2.5 μm < Dp < 10 μm) have been associated more strongly than PM2.5 with respiratory hospital admissions (Brunekreef & Forsberg, 2005). Ultrafine particles (Dp < 0.1 μm) have been suggested to pose a great risk to human health due to their high number concentration in urban environments and ability to penetrate into the blood circulation (Delfino et al., 2005).

There have been relatively few previous animal studies comparing the toxic properties of urban air coarse, fine, and ultrafine particles or addressing the issue of regional, seasonal, and source-related heterogeneities in the toxic properties of these particles. This kind of studies would support the development of better targeted abatement and monitoring strategies, because the current health-based ambient air monitoring and regulation all over the world are mainly based on PM10 or PM2.5 mass concentration, assuming all source contributions equally toxic. Dick et al. (2003) have shown variations in inflammatory activity of urban air particulate samples collected in rather unconventional size ranges (Dp: <1.7 μm, 1.7–3.5 μm, and 3.5–20 μm) in Research Triangle Park, NC. There is also evidence that PM10-2.5 and PM2.5-0.1 samples collected in four European cities during three seasons evoke adjuvant activity of varying strength in a mouse allergy model (Steerenberg et al., 2004). Moreover, there has been seasonal variation in the inflammatory activity of these particles in vitro (Dybing et al., 2004; Hetland et al., 2005). In contrast to the epidemiological findings, coarse particulate samples have consistently shown much higher in vivo inflammatory activities per unit of mass than the fine particulate samples. However, only few studies have considered a possibility of different time courses for the measured response parameters in the rodent lungs (Gerlofs-Nijland et al., 2005; Walters et al., 2001), and none of these have examined the issue of time course in relation to more than one particulate size range.

In the PAMCHAR project, we investigate European contrasts in the toxicological characteristics of PM10-2.5, fine (PM2.5-0.2; 0.2 μm < Dp < 2.5 μm), and ultrafine (PM0.2; Dp < 0.2 μm) particles sampled with an optimized high-volume cascade impactor (Sillanpää et al., 2003). Six sampling sites across Europe were chosen to represent different source environments and seasons of public health interest. The objectives of the present study were: (1) to investigate in the healthy mouse lung the dose responses of inflammatory responses to these particulate samples at a commonly used time point of 24 h after intratracheal instillation, (2) to compare the time course of acute inflammatory response in different parameters measured from the bronchoalveolar lavage fluid (BALF), and (3) to examine the source associations with possible heterogeneities observed in the inflammatory activities of particulate samples between the campaigns. We used well-established biochemical markers of cytotoxicity (lactate dehydrogenase [LDH]), microvascular leakage [total protein concentration]) and inflammation (total cell number, cell differentials, cytokines) in the analysis of BALF. The cytokines that were measured included the proinflammatory tumor necrosis alpha (TNF-α) and interleukin-6 (IL-6) as well as a chemokine, keratinocyte-derived chemokine (KC).

MATERIAL AND METHODS
Sampling Campaigns and Particulate Sources
Samples of particulate matter were collected in a series of sampling campaigns from six European cities in selected seasons: Duisburg autumn (4 October–21 November 2002), Prague winter (29 November 2002–16 January 2003), Amsterdam
winter (24 January–13 March 2003), Helsinki spring (21 March–12 May 2003), Barcelona spring (28 March–19 May 2003), and Athens summer (2 June–21 July 2003). The campaign periods included seasons with high particulate concentrations and suspected enhanced health effects in the populations. The sampling sites were located in urban background areas as previously described in detail (Sillanpää et al., 2005). The same sampling station and samplers were used in all the campaigns with the exception of Helsinki, where another identical set of instruments was used.

Chemical mass closure of particulate samples collected with dichotomous virtual impactors has shown that the major components of PM$_{2.5}$ in our sampling campaigns are carbonaceous compounds (organic matter + elemental carbon) and secondary inorganic ions (SO$_4^{2-}$, NO$_3^-$, NH$_4^+$), whereas those of PM$_{10-2.5}$ are crustal material, carbonaceous compounds, sea salt, and nitrate (Sillanpää et al., 2006). Organic matter has a large contribution to PM$_{2.5}$ (21–54% of total mass) and its sources have also been analyzed with chemical tracers (Sillanpää et al., 2005). Automotive traffic has the highest impact on fine particulate organic matter in Duisburg and Barcelona, followed by Amsterdam and Athens. In addition, the source tracers have revealed substantial contributions from metal industries (Duisburg > Barcelona), coal combustion (Prague > Duisburg > Amsterdam > Barcelona), fuel oil combustion—most likely in ships at city harbor (Barcelona > Helsinki > Amsterdam), and biomass combustion (Prague > Amsterdam > Duisburg). Finally, photochemical processes in the atmosphere have been suggested to make the highest contribution to fine particulate organic matter in Barcelona followed by Athens and Helsinki.

The source contributions to the smaller but still substantial organic matter of PM$_{10-2.5}$ (9–27% of total mass) in our campaigns have not been as well determined as for PM$_{2.5}$ (Sillanpää et al., 2005). Part of that is likely to originate from local sources of incomplete coal and biomass combustion, and a smaller part is probably from traffic exhausts and oxidation of volatile organic compounds. This is suggested by the co-appearance of higher contributions of elemental carbon to PM$_{10-2.5}$ in autumn and winter campaigns (2.3–5.5%) compared to spring and summer campaigns (0.96–1.6%) (Sillanpää et al., 2006). Other major sources include biological debris, pollens, fungal spores, etc.

The particulate samples for toxicological studies were collected in 3- to 4-d periods per week in each city. The collection was made with a modified Harvard high volume cascade impactor (HVCI) to enable a high efficiency, representative sampling of particulate mass in different size ranges (Sillanpää et al., 2003). The PM$_{10-2.5}$ and PM$_{2.5-0.2}$ samples ($n = 14$) were collected on polyurethane foam (PUF; antistatic polyurethane foam 87035K13, McMaster-Carr, New Brunswick, NJ), while the PM$_{0.2}$ samples were collected on glass fiber filters (Munktell MGA, Munktell Filter AB, Grycksbo, Sweden). All the particulate samples were stored at −20°C after collection with the HVCI. Thereafter, they were shipped frozen to the National Public Health Institute, Kuopio, Finland.

A summary of air quality during the campaign periods is presented in Table 1. The average solar radiation and ozone concentration during the spring and summer campaigns of Helsinki, Barcelona, and Athens were much higher than those during the autumn and winter campaigns of Duisburg, Prague, and Amsterdam.

### Sample Preparation for Animal Studies

The size-segregated particulate samples were prepared for the animal experiments using previously validated procedures (Jalava et al., 2005, 2006). Briefly, the sampled PUF strips or

**TABLE 1**

Means of mass concentrations of urban air particulate matter in three HVCI size ranges and of gaseous pollutant concentrations and meteorological parameters during the sampling campaigns in six European cities

<table>
<thead>
<tr>
<th></th>
<th>Duisburg autumn</th>
<th>Prague winter</th>
<th>Amsterdam winter</th>
<th>Helsinki spring</th>
<th>Barcelona spring</th>
<th>Athens summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM$_{10-2.5}$ (µg/m$^3$)</td>
<td>7.6</td>
<td>5.9</td>
<td>9.8</td>
<td>12.8</td>
<td>22.9</td>
<td>29.6</td>
</tr>
<tr>
<td>PM$_{2.5-0.2}$ (µg/m$^3$)</td>
<td>15.8</td>
<td>25.1</td>
<td>22.8</td>
<td>8.3</td>
<td>14.3</td>
<td>18.9</td>
</tr>
<tr>
<td>PM$_{0.2}$ (µg/m$^3$)</td>
<td>2.8</td>
<td>4.7</td>
<td>3.8</td>
<td>2.7</td>
<td>4.5</td>
<td>6.7</td>
</tr>
<tr>
<td>CO (mg/m$^3$)</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
<td>0.3</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>NO$_2$ (µg/m$^3$)</td>
<td>34</td>
<td>33</td>
<td>43</td>
<td>24</td>
<td>43</td>
<td>37</td>
</tr>
<tr>
<td>O$_3$ (µg/m$^3$)</td>
<td>17</td>
<td>21</td>
<td>22</td>
<td>63</td>
<td>49</td>
<td>85</td>
</tr>
<tr>
<td>SO$_2$ (µg/m$^3$)</td>
<td>10</td>
<td>16</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>T (°C)</td>
<td>9</td>
<td>−2</td>
<td>4</td>
<td>4</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>RH (%)</td>
<td>88</td>
<td>87</td>
<td>82</td>
<td>67</td>
<td>64</td>
<td>43</td>
</tr>
<tr>
<td>Rain (mm)</td>
<td>88</td>
<td>47</td>
<td>60</td>
<td>40</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Radiation (W/m$^2$)</td>
<td>42</td>
<td>n.a</td>
<td>68</td>
<td>157</td>
<td>225</td>
<td>320</td>
</tr>
</tbody>
</table>

*Note.* n.a., Not available due to missing data. The largest value for each parameter is in boldface.
quarters of glass fiber filter were extracted with methanol (J. T. Baker HPLC grade, Deventer, The Netherlands) for 2 × 30 min in a water bath sonicator (FinnSonic m20, FinnSonic Oy, Lahti, Finland) at 20°C. The methanol extracts from the particle-loaded substrates of each city were pooled together by size range, and the excess methanol was evaporated at +35°C with a rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany) that was attached to a vacuum pump (Vacuubrand CVC 2000, Wertheim, Germany) set at 150 mbar. Thereafter, the methanol suspension containing PM$_{0.2}$ particles was filtered (Schleicher & Schuell FP 30/0.2 CA-S filter, pore size 0.2 μm, Dassel, Germany) to remove glass fibers derived from filters. The concentrated suspension was divided into 10-ml glass tubes as defined amounts of particulate mass and dried under nitrogen (99.5%) flow. Samples were stored in freezer at −20°C until prepared for animal exposures. A similar procedure to the size-segregated particulate samples was used in preparation of the corresponding pooled blanks (Jalava et al., 2006).

The overall mean extraction efficiency of particulate matter from the PUF substrates was 85% (range 61–99%), with a tendency toward a higher efficiency with samples in smaller particulate size ranges. The extraction efficiency could not be measured for the PM$_{0.2}$ particles collected on glass fiber filters.

For animal exposures, the dry particulate samples and blanks were taken from −20°C. Closed sample tubes were allowed to warm up to room temperature for 10 min before adding 1 ml pathogen-free water (Sigma) per 10 mg particulate mass. Thereafter, the samples were suspended and sonicated at +20°C for 30 min in a water bath sonicator (Finnsonic m03, Finnsonic Oy, Lahti, Finland). The suspension was diluted into pathogen-free water (Sigma) to obtain final concentrations of 0.5, 1.5, and 5 mg/ml to be used in animal exposures. The blank samples were diluted in an equal volume of pathogen-free water (Sigma) to assure that the vehicle of particulate suspension and possible impurities in methanol extraction were not sources of toxic activity. Pathogen-free water was used as the vehicle, because the concentrated suspensions of particulate samples contained substantial amounts of cations and anions (e.g., sea salt) and we did not want to modify their chemical compositions. Use of saline would have made the suspensions hyperosmolar.

Animals
A well-established mouse model (Jussila et al., 2001) was used due to a close resemblance of the immunology of this species to humans and a low consumption of particulate mass in intratracheal instillations. Pathogen-free male C57BL/6J mice 8–9 wk old (weight 19–30 g) were obtained from the breeding colony of the National Public Health Institute, Department of Environmental Health, Kuopio, Finland. They were transferred from a barrier unit to a conventional animal room 2 wk before the experiment. After a 1-wk acclimatization period, the animals were transferred into metal cages and they were housed singly on aspen wood chips (FinnTapvei, Finland). The mice received water and R36 maintenance diet (Lactamin, Stockholm, Sweden) ad libitum. The animals were kept on a 12-h light/dark rhythm (7 a.m. to 7 p.m.) at room temperature (21°C) and 21–63% humidity.

Experimental Designs
The first part of our study is a dose-response screening of the six-city particulate samples in three size ranges, while the second part consists of a time dependency investigation of different parameters in the acute inflammatory response to a selected single dose. The best time points were identified for each measured parameter to enable a reliable comparison of the inflammatory activities of size-segregated particulate samples between the sampling campaigns.

In the dose-response study, the mice were exposed to a single dose of particulate samples via intratracheal instillation (50 μl/animal), using 1-, 3-, and 10-mg/kg dose levels. The dose range was selected on the basis of a pilot study and previously published studies in rodents. In the PM$_{10-2.5}$ and PM$_{2.5-0.2}$ size ranges, the bronchoalveolar lavage fluids (BALF) of mice (n = 5–8) were collected at 24 h after the exposure. In the PM$_{0.2}$ size range, an earlier 12-h time point was used for BALF collection (n = 2–3) on the basis of a pilot study that showed larger responses at that time point than at 24 h. As control groups, we used untreated mice as well as animals treated with pathogen-free water (Sigma) or solution extracted from blank filters (negative controls). Ottawa dust (EHC-93) was used as a positive control of the intratracheal instillation technique in the dose-response study, because it is a well-documented reference material that induces inflammatory responses (Vincent et al., 1997).

In the time-course study, the mice were intratracheally exposed to a single particulate dose of 10 mg/kg (50 μl/animal). In experiments on PM$_{10-2.5}$ and PM$_{2.5-0.2}$ samples, the BALF of mice (n = 5–6) was collected at 4, 12, or 24 h after the exposure. In experiments on PM$_{0.2}$ samples, BALF was collected at 4 (n = 5–6) or 12 h (n = 2–3).

Intratracheal Instillation
The animals were anesthetized with vaporized 4.5% sevoflurane (Sevorane, Abbott, IL) and positioned in a 66° upward bended position with the incisors placed on a thin wire. A cold-light source (KL 1500 electronic, Schott, Germany) was placed against the throat. The dosing of particulate suspension was performed under visual control, while the tongue was pulled out with forceps to prevent the mouse from swallowing. The samples were delivered onto the vocal folds with a Finn pipette tip (Finntip 200 Ext, Thermo Electron Oy, Vantaa, Finland) and, immediately thereafter, the nostrils were covered, thus enforcing the mouse to inspire the instilled particulate suspension.
Bronchoalveolar Lavage

At the defined time point, the mice were anesthetized with pentobarbital (60 mg/kg) and exsanguinated by cardiac puncture. The lungs were perfused with sterile saline. Tracheas were cannulated with polyethylene tubing and the lungs were lavaged with two portions of sterile saline (30 ml/kg), three times each. These two portions of BALF were combined and kept on ice. Cells were separated from the BALF by centrifugation (1800 rpm, 10 min; Hettich universal 30F, Hettich-zentrifugen, D-7200 Tuttlingen, Germany). The supernatant was removed and the cells were resuspended in 220 μl sterile saline for counting the total cell number in each BALF sample by using the Trypan blue exclusion method. The rest of cell suspension was used in cytoospin (210 μl, 500 rpm, 8 min; Megafuge, Heraeus Instruments, Germany). Slides were fixed in methanol and stained with May–Grünwald–Giemsa dye. For cell differential, 100 cells were counted from 3 different views of the slide and an average of these was taken as the percentage amount of cell type. The supernatant remaining after LDH and total protein analysis was frozen (−80°C) for cytokine analysis.

Biochemical Analyses

Lactate dehydrogenase (LDH) and total protein concentration were analyzed from fresh supernatants. LDH was analyzed by using a cytotoxicity detection kit (Roche Diagnostics GmbH, Germany) with minor modifications as earlier described by Jussila et al. (2001). Total protein was analyzed by a modified DC protein assay (Bio-Rad, Hercules, CA) as earlier described in detail by Jussila et al. (2001). Tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and keratinocyte-derived chemokine (KC) were analyzed from BALF. All immunochemical analyses were made using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) according to manufacturer’s instructions as earlier described in detail by Jalava et al. (2005).

Statistical Methods

All the measured values were first analyzed with Levene’s test for equality of variances. Statistical differences in the measured BALF parameters between the particulate sample-treated animals and blank sample-treated animals (controls) were determined with analysis of variance (ANOVA) and Dunnett’s test. In cases where Levene’s test gave values <.05, the Kruskal–Wallis test was used. The differences were regarded as statistically significant at $p < .05$. Differences between sampling sites were tested with Levene’s test for equality of variances followed by Tukey’s HSD or Dunnett’s C test.

RESULTS

The responses to particulate samples are described by size range for each parameter and the comparisons between samples of different campaigns are made on equal mass basis. Blank sample–treated mice showed negligible signs of inflammation in their BALF parameters like the unexposed and vehicle-treated control animals.

Dose Relationship of Particulate Responses

Total cell number. Figure 1A shows the dose dependency of total cell number responses in BALF to the size-segregated particulate samples from six cities. The total cell number was not statistically significantly increased at the lowest dose (1 mg/kg) of any of the three particulate size ranges. The dose of 3 mg/kg increased cell numbers (mean ± SE) significantly only in the PM10-2.5 size range (up to 1.5 ± 0.3 × 10^6 cells/ml). The highest dose of the PM10-2.5 samples (10 mg/kg) induced even larger significant increases (up to 2.6 ± 0.3 × 10^6 cells/ml) compared to the control (0.43 ± 0.03 × 10^6 cells/ml). In the PM2.5-0.2 size range, only the samples from Amsterdam and Athens caused significant responses (up to 1.5 ± 0.4 × 10^6 cells/ml) at the highest dose of 10 mg/kg. None of the PM0.2 samples affected total cell number in BALF even at the highest dose.

Total protein and LDH. The dose-related total protein concentrations in BALF induced by the size-segregated particulate samples are shown in Figure 1B. There were no statistically significant increases at the lower doses of 1 mg/kg and 3 mg/kg of the samples in any size range, excluding the PM0.2 sample from Athens. At 10 mg/kg, all the six particulate samples in the PM10-2.5 size range increased total protein concentration significantly (up to 393 ± 58 μg/ml). In the PM2.5-0.2 size range, the samples from Duisburg, Prague, Barcelona, and Athens induced significantly higher protein levels (up to 324 ± 51 μg/ml) than the control (203 ± 15 μg/ml). In contrast, the samples in the PM0.2 size range had no effect even at the highest dose of 10 mg/kg. Particulate samples in all three size ranges induced small, inconsistent changes in LDH (data not shown).

Cytokine concentrations. Figure 2, A–C, shows the dose dependency of TNF-α, IL-6, and KC responses in BALF to the size-segregated particulate samples from the six cities. Only the particulate samples in the PM10-2.5 size range induced dosee-dependent response patterns, mostly with statistical significances at the highest dose of 10 mg/kg. Even these increases in cytokine concentrations were small when compared to the corresponding controls: up to sixfold with TNF-α, sixfold with IL-6, and fivefold with KC. The samples in the smaller size ranges of PM2.5-0.2 and PM0.2 caused only minimal, inconsistent responses.

Time Dependency of PM Effects

Total cell number. The time courses of effects on total cell number in BALF, caused by the size-segregated particulate samples from the six cities, are shown in Figure 3. The cell number was not increased at 4 h after exposure to any sample. In the PM10-2.5 size range, all 6 samples caused significant increases at the 12-h time point when compared to the control (0.8 ± 0.2 × 10^6 cells/ml). The Helsinki sample induced the smallest response (2.1 ± 0.1 × 10^6 cells/ml), which differed significantly from the largest response (3.8 ± 0.4 × 10^6 cells/ml),
produced by the Amsterdam sample. At 24 h after exposure, the total cell numbers were lower than at 12 h, but they were still significantly higher than the corresponding control in every case. The difference was most prominent with the Amsterdam sample, with the total cell number being at 24 h \( (2.1 \pm 0.1 \times 10^6 \text{ cells/ml}) \) about half of that detected at 12 h (Figure 3A).

In the PM\(_{2.5-0.2}\) size range, all the samples, excluding Amsterdam, induced their highest total cell numbers at 12 h after the exposure. At that time point, the increase in BALF cell number by the samples from Helsinki, Barcelona, and Athens was statistically significant when compared to the control \( (0.9 \pm 0.2 \times 10^6 \text{ cells/ml}) \). The Amsterdam sample induced the smallest response \( (1.2 \pm 0.3 \times 10^6 \text{ cells/ml}) \), which was significantly different from the largest response \( (2.8 \pm 0.1 \times 10^6 \text{ cells/ml}) \), produced by the Athens sample. Only the Amsterdam sample caused its largest, statistically significant response at 24 h after exposure \( (1.5 \pm 0.1 \times 10^6 \text{ cells/ml}) \) when compared to the control. With all the other samples, the total cell numbers at 24 h were much lower than those at 12 h (Figure 3B).

In the PM\(_{0.2}\) size range, the particulate samples other than that from Athens caused no significant increase in BALF total cell number (Figure 3C). Even the largest responses to the PM\(_{0.2}\) samples at 12 h were negligible when compared to the particulate samples in larger size ranges. The 24-h time point was not tested with the PM\(_{0.2}\) samples.

**FIG. 1.** Total cell number (A) and total protein (B) in BALF at 24 h (PM\(_{10-2.5}\)) and 12 h (PM\(_{0.2}\)) after intratracheal exposure of healthy C57Bl/6J mice to a single dose (1, 3, or 10 mg/kg) of particulate samples in 3 size ranges or to a corresponding blank sample. Each bar shows mean ± SE \( (n = 5–8, \text{ except PM}_{0.2} \text{ at } 12 \text{ h } n = 2–3) \). Asterisk indicates statistically significant difference from the control (Dunnett, \( p < .05 \)). (Continued)
Cell differential. Figure 4 shows the cell differentials in BALF at 12 h after exposure to the PM$_{10-2.5}$ and PM$_{2.5-0.2}$ samples from the 6 sampling campaigns. All the PM$_{10-2.5}$ samples induced large, statistically significant increases in neutrophil number concentration when compared to the control ($0.4 \pm 0.4 \times 10^5$ cells/ml). The Helsinki sample produced the lowest number concentration ($1.5 \pm 0.1 \times 10^6$ cells/ml), which differed significantly from the responses to the samples from Amsterdam ($2.8 \pm 0.3 \times 10^6$ cells/ml) and Barcelona ($2.4 \pm 0.3 \times 10^6$ cells/ml). None of the PM$_{10-2.5}$ samples increased macrophage number concentration, but the Prague sample caused a significantly lower number ($3.6 \pm 0.4 \times 10^5$ cells/ml) than the control ($7.8 \pm 1.8 \times 10^5$ cells/ml). The PM$_{10-2.5}$ samples from Amsterdam, Barcelona, and Athens increased slightly but statistically significantly the number of lymphoplasmacytic cells from control ($1.8 \pm 0.1 \times 10^4$ cells/ml) (Figure 4A).

The PM$_{2.5-0.2}$ samples from Athens, Barcelona, and Helsinki induced about as high increases in BALF neutrophil number as the respective PM$_{10-2.5}$ samples. In contrast, the responses to the PM$_{2.5-0.2}$ samples from Duisburg, Prague and Amsterdam were much smaller than those to the respective PM$_{10-2.5}$ samples. All the PM$_{2.5-0.2}$ samples, excluding Amsterdam, increased the neutrophil number concentration statistically significantly when compared to the control ($0.3 \pm 0.1 \times 10^5$ cells/ml). The highest neutrophil number induced by the Athens sample ($2.1 \pm 0.4 \times 10^6$ cells/ml) differed significantly from the lowest number induced by the Amsterdam sample ($0.6 \pm 0.1 \times 10^6$ cells/ml). None of the PM$_{2.5-0.2}$ samples changed macrophage number concentration, and the number of lymphoplasmacytic cells was slightly increased only by the Barcelona sample (Figure 4B).

Total protein and LDH. The time courses of effects on total protein concentration in BALF, caused by the size-segregated
particulate samples from the six cities, are shown in Figure 5. None of the particulate samples increased protein concentration significantly at 4 h after exposure.

All the PM$_{10-2.5}$ samples, excluding Prague, induced significantly higher protein concentration at the 12-h time point than the control ($183 \pm 11 \, \mu g/ml$). The Prague sample produced the smallest response ($250 \pm 12 \, \mu g/ml$), which was significantly different from the largest response ($421 \pm 8 \, \mu g/ml$), induced by the Duisburg sample, as well as from the responses induced by the Barcelona, Amsterdam, and Athens samples. At 24 h after exposure, the total protein concentrations in BALF, caused by the Prague, Amsterdam, and Barcelona samples, were significantly higher than the control level. The Prague sample produced a clearly higher protein concentration ($390 \pm 32 \, \mu g/ml$) at 24 h than at 12 h, while an opposite response pattern was seen with the Duisburg sample. The responses to the Amsterdam, Helsinki, Barcelona, and Athens samples were roughly similar at the two time points (Figure 5A).

In the PM$_{2.5-0.2}$ size range, the Barcelona ($389 \pm 31 \, \mu g/ml$) and Athens ($421 \pm 78 \, \mu g/ml$) samples significantly increased total protein concentration in BALF at 12 h after exposure, when compared to the control ($231 \pm 46 \, \mu g/ml$). The Prague sample induced its largest response ($312 \pm 44 \, \mu g/ml$) at the 24-h time point (Figure 5B). The PM$_{0.2}$ samples caused minimal, inconsistent effects on total protein concentration in BALF at 12 h (Figure 5C), and the responses at the 24-h time point were not tested.

The particulate samples in all the size ranges caused only small, inconsistent changes in LDH measured at 4, 12 and 24 h after exposure (data not shown).

**FIG. 2.** The TNF-α (A), IL-6 (B) and KC (C) concentrations in BALF at 24 h (PM$_{10-2.5}$ and PM$_{2.5-0.2}$) and 12 h (PM$_{0.2}$) after intratracheal exposure of healthy C57Bl/6J mice to a single dose (1, 3, or 10 mg/kg) of particulate samples in 3 size ranges or to a corresponding blank sample. Each bar shows mean ± SE ($n = 5–8$, except PM$_{0.2}$ at 12 h $n = 2–3$). Asterisk indicates statistically significant difference from the control (Dunnett, $p < .05$). (Continued)
**TNF-α production.** The time courses of effects on TNF-α concentration in BALF, caused by the size-segregated particulate samples from the six cities, are shown in Figure 6. All the PM_{10-2.5} samples induced their highest TNF-α concentrations at 4 h after exposure, and all the responses, except for that to the Duisburg sample, were statistically significantly higher than the corresponding control (14 ± 2 pg/ml). The Barcelona sample induced the largest 130-fold increase in TNF-α concentration (1812 ± 55 pg/ml), which was also significantly higher than the smallest response (620 ± 147 pg/ml) to the Duisburg sample. At the 12-h time point, all the responses were much smaller (range 145–299 pg/ml) than at 4 h, although they were still significantly higher than the control. At 24 h, the concentrations (44–137 pg/ml) were near the control level (Figure 6A).

In the PM_{2.5-0.2} size range, 3 samples out of a total of 6 significantly increased TNF-α concentration at 4 h after exposure, when compared to the control (14 ± 1 pg/ml). These samples were from Amsterdam (185 ± 113 pg/ml), Barcelona (263 ± 33 pg/ml), and Athens (398 ± 34 pg/ml). The responses to the Barcelona and Athens samples were also significantly larger than the smallest response to the Prague sample (40 ± 4 pg/ml). All the responses to the PM_{2.5-0.2} samples were minimal (20–48 pg/ml) at 12 and 24 h after exposure (Figure 6B), and none of the PM_{0.2} samples significantly increased TNF-α concentration in BALF (Figure 6C).

**IL-6 production.** Figure 7 shows the time courses of effects on IL-6 concentration in BALF caused by the size-segregated particulate samples. All the PM_{10-2.5} samples induced their highest IL-6 concentrations at 4 h after exposure. The concentrations were significantly higher than the control (59 ± 6 pg/ml). The responses to the samples from Athens (1525 ± 72 pg/ml) and Barcelona (1329 ± 160 pg/ml) were also significantly larger than
the smallest response to the Prague sample (786 ± 76 pg/ml). At the 12-h time point, the responses were clearly smaller (164–380 pg/ml) but still significantly higher than the controls. At the latest 24-h time point, all the IL-6 concentrations (range 60–135 pg/ml) were near the control level (Figure 7A).

In the PM2.5-0.2 size range, there were large differences in IL-6 concentrations induced by the particulate samples. At 4 h after exposure, the responses to the samples from Duisburg, Helsinki, Barcelona, and Athens were significantly larger than for the control (59 ± 9 pg/ml). Moreover, the responses to the samples from Barcelona (843 ± 96 pg/ml) and Athens (1258 ± 115 pg/ml) were significantly larger than the smallest response, to the Prague sample (216 ± 25 pg/ml). All the responses to the PM2.5-0.2 samples at 12- and 24-h time points were minimal (40–106 pg/ml) (Figure 7B).

In the PM0.2 size range, the Helsinki sample induced a small but statistically significantly higher IL-6 concentration (342 ± 89 pg/ml) than the control (19 ± 5 pg/ml) at 4 h after the exposure. This response was also significantly larger than the smallest response to the Barcelona sample (32 ± 6 pg/ml). However, the overall IL-6 responses to the PM0.2 samples were negligible when compared to the samples in the PM10-2.5 and PM2.5-0.2 size ranges. Responses to the PM0.2 samples were minimal at 12 h after exposure (Figure 7C).

**KC production.** The time courses of effects on KC concentration in BALF, caused by the size-segregated particulate samples from the six sampling campaigns, are shown in Figure 8. All the PM10-2.5 samples induced their highest KC concentrations at 4 h after the exposure, differing statistically significantly from the control (29 ± 2 pg/ml). There were no significant differences in KC responses (678–962 pg/ml) between the samples from the six cities. At the 12-h time point, the response to the Prague sample (654 ± 123 pg/ml) was still almost at the same level as at 4 h, whereas the responses to all other PM10-2.5
FIG. 3. The total cell number in BALF at 4, 12, and 24 h (PM_{10-2.5} and PM_{2.5-0.2}) and 12 h (PM_{0.2}) after intratracheal exposure of healthy C57Bl/6J mice to a single dose (10 mg/kg) of particulate samples in 3 size ranges or to a corresponding blank sample. Each bar shows mean ± SE (n = 5–8, except PM_{0.2} at 12 h n = 2–3). The arrow indicates a statistically significant difference from the smallest response at the most appropriate time point of 12 h (Tukey, p < .05). Asterisk indicates statistically significant difference from the control (Dunnett, p < .05).

The samples (209–506 pg/ml) were smaller than those measured at 4 h. At 24 h after exposure, the KC concentrations in BALF (23–117 pg/ml) were near the control level (Figure 8A).

In the PM_{2.5-0.2} size range, all the samples, except for that from Prague, induced the highest KC concentration at 4 h after exposure. The samples from Athens (772 ± 78 pg/ml), Barcelona (575 ± 95 pg/ml), and Helsinki (431 ± 105 pg/ml) induced significantly larger responses than the control (24 ± 7 pg/ml). The responses to the Athens and Barcelona samples were also significantly larger than the smallest response to the Prague sample (112 ± 12 pg/ml). At the 12-h time point, all the responses (76–137 pg/ml) in the PM_{2.5-0.2} size range were near the control level (28 ± 14 pg/ml), with the exception that the response to the Prague sample was higher than at 4 h. None of the PM_{2.5-0.2} samples increased KC concentration (31–36 pg/ml) significantly at 24 h after exposure (Figure 8B).
FIG. 4. Cell differentials in BALF at 12 h after intratracheal exposure of healthy C57Bl/6J mice to a single dose (10 mg/kg) of particulate samples in 2 size ranges or to a corresponding blank sample. Each bar shows the mean of 5–6 mice. Asterisk indicates statistically significant difference from the control (Dunnett, \( p < .05 \)).

In the PM_{0.2} size range, there were only occasional small increases in KC concentration (35–232 pg/ml) at 4 h after exposure. None of the samples significantly increased KC concentration at 12 h. The 24-h time point was not tested with the PM_{0.2} samples (Figure 8C).

DISCUSSION

There were multiple heterogeneities in the inflammatory activities of the ambient air coarse, fine, and ultrafine particulate samples with regard to both the magnitude and time course of the responses. It should be noted, however, that the present findings do not specifically represent any city or season due to the short sampling periods. They rather represent toxic activities of complex mixtures of size-segregated particulate matter in selected source environments variably affected by common local emission sources and seasonal factors.

Dose Dependency of Pulmonary Responses

We showed clear dose-dependent increases in BALF total cell number, and a similar trend in total protein concentration, at 24 h after intratracheal instillation of the PM_{10-2.5} samples. In contrast, only the highest dose (10 mg/kg) of the PM_{2.5-0.2} samples caused occasional significant responses, and no consistent response pattern was found to any PM_{0.2} sample.

The present inflammatory findings in the mouse lung are in line with the study of Gerlofs-Nijland et al. (2005) in spontaneously hypertensive rats, showing statistically significant increases in BALF total cell number. However, these authors did
FIG. 5. The total protein concentration in BALF at 4, 12, and 24 h (PM$_{10-2.5}$ and PM$_{2.5-0.2}$) and 12 h (PM$_{0.2}$) after intratracheal exposure of healthy C57Bl/6J mice to a single dose (10 mg/kg) of particulate samples in 3 size ranges or to a corresponding blank sample. Each bar shows mean ± SE (n = 5–8, except PM$_{0.2}$ at 12 h n = 2–3). The arrow indicates a statistically significant difference from the smallest response at the most appropriate time point of 12 h (Tukey, p < .05). Asterisk indicates statistically significant difference from the control (Dunnett, p < .05).

not find increases in total protein concentration at 24 h after intratracheal instillation of the doses 3 and 10 mg/kg of road tunnel dust PM$_{10}$ samples. In contrast, Dick et al. (2003) have reported a significant but not dose-dependent response in BALF neutrophils of mice to urban air coarse particulate samples (PM$_{20-3.5}$; 0.4–4 mg/kg), while their PM$_{3.5-1.7}$ and PM$_{1.7}$ samples increased neutrophil numbers in a significant, dose-related manner. This apparent disagreement with our present results may, at least partly, be due to use of particulate samples with different size cutoff diameters. Dick et al. (2003) have found no statistically significant increase in BALF total protein concentration. However, Ulrich et al. (2002) have reported in ozone preexposed rats that Ottawa dust (EHC-93) induces a significant increase in BALF total protein concentration at 48 h after a relatively large intratracheal dose of approximately 25 mg/kg. Thus, BALF total cell number seems to be more sensitive than protein concentration as an indicator of particulate-induced acute inflammatory response in the rodent lung.

There was a tendency toward small dose-dependent increases in all the three cytokine (TNF-α, IL-6, KC) concentrations in BALF at 24 h after exposure to the PM$_{10-2.5}$ samples, whereas the responses to the PM$_{2.5-0.2}$ and PM$_{0.2}$ samples were negligible or inconsistent. For comparison, Dick et al. (2003) have reported significantly increased IL-6 concentrations in BALF of mice...
FIG. 6. TNF-α concentration in BALF at 4, 12, and 24 h (PM_{10-2.5} and PM_{2.5-0.2}) and 12 h (PM_{0.2}) after intratracheal exposure of healthy C57Bl/6J mice to a single dose (10 mg/kg) of particulate samples in 3 size ranges or to a corresponding blank sample. Each bar shows mean ± SE (n = 5–8, except PM_{0.2} at 12 h n = 2–3). The arrow indicates a statistically significant difference from the smallest response at the most appropriate time point of 4 h (Tukey, p < .05). Asterisk indicates statistically significant difference from the control (Dunnett, p < .05).

at 18 h after an intratracheal dose of approximately 4 mg/kg of the particulate samples in three size ranges. Moreover, there have been statistically significant increases in rat BALF cytokine concentrations at 24 h after intratracheal instillation of 3 and 10 mg/kg of road tunnel dust PM_{10} and EHC-93 in spontaneously hypertensive rats (Gerlofs-Nijland et al., 2005).

Overall, cytokine responses to ambient air particulate samples have been small but most often statistically significant at 18–24 h after intratracheal instillation of particulate doses close to our highest dose. Systematic differences in water solubility of particulate material in the three size ranges may contribute to the observed differences in inflammatory activity. The chemical mass closure study of Sillanpää et al. (2005) on low-volume virtual impactor samples of the same sampling campaigns suggests that our PM_{10-2.5} samples contain a much larger portion of insoluble inorganic material than the PM_{2.5-0.2} and PM_{0.2} samples. This may facilitate particle uptake by inflammatory cells like macrophages (Kendall et al., 2002).
DOSE AND TIME DEPENDENCY OF URBAN AIR PM

FIG. 7. IL-6 concentration in BALF at 4, 12, and 24 h (PM_{10-2.5} and PM_{2.5-0.2}) and 12 h (PM_{0.2}) after intratracheal exposure of healthy C57Bl/6J mice to a single dose (10 mg/kg) of particulate samples in 3 size ranges or to a corresponding blank sample. Each bar shows mean ± SE (n = 5–8, except PM_{0.2} at 12 h n = 2–3). The arrow indicates a statistically significant difference from the smallest response at the most appropriate time point of 4 h (Tukey, p < .05). Asterisk indicates statistically significant difference from the control (Dunnett, p < .05).

Time Dependency of Pulmonary Responses

Consideration of the time dependency of the pulmonary responses led to a very different overall picture of the inflammatory activity of the size-segregated particulate samples compared to our findings at a single time point of 24 h after exposure in the dose-response study.

Both the PM_{10-2.5} and PM_{2.5-0.2} samples generally induced their largest increases in BALF total cell number at 12 h after intratracheal instillation. The cell numbers were still significantly increased at 24 h with the PM_{10-2.5} samples, whereas that was not the case for the PM_{2.5-0.2} samples except for Amsterdam. We found no increases in total cell number at the earliest 4-h time point with the samples of any size range, which has also been the finding in the rat study of Gerlofs-Nijland et al. (2005). In contrast, it has been shown that neutrophil numbers in rat BALF are significantly increased already at 6 h after exposure to PM_{10} collected in Edinburgh (Li et al., 1996). Walters et al. (2001) have used 8 time points from 6 h to 14 days and report
FIG. 8. KC concentration in BALF at 4, 12, and 24 h (PM$_{10-2.5}$ and PM$_{2.5-0.2}$) and 12 h (PM$_{0.2}$) after intratracheal exposure of healthy C57Bl/6J mice to a single dose (10 mg/kg) of particulate samples in 3 size ranges or to a corresponding blank sample. Each bar shows mean ± SE ($n$ = 5–8, except PM$_{0.2}$ at 12 h $n$ = 2–3). The arrow indicates a statistically significant difference from the smallest response at the most appropriate time point of 4 h (Tukey, $p$ < .05). Asterisk indicates statistically significant difference from the control (Dunnett, $p$ < .05).

We observed that the increases in BALF total cell number concentration were largely due to greatly increased numbers of neutrophils, which has also been a consistent finding in several previous studies on urban air particulate exposures in mice and rats (Adamson & Prieditis, 2004; Gavett et al., 2003; Gerlofs-Nijland et al., 2005; Schins et al., 2003). However, in A/J mice, the increase in BALF total cell number has been largely due to increased eosinophil numbers (Walters et al., 2001), suggesting a possibility of significant variations between different mouse strains. Some increases in BALF total cell number of mice at 6 h with the largest response at 12 h after particulate exposure. Schins et al. (2003) have used only a single time point of 18 h in their rat study and report small responses in total cell number to fine particulate samples. Thus, 12 h after intratracheal exposure of mice to ambient air particulate samples seems a more appropriate time point than 24 h for assessment of the acute inflammatory response in BALF total cell number. This may also apply to rat studies.
strains. The macrophage numbers were most often decreased at 12 h, which could be due to their tighter adhesion to the alveolar epithelium, as suggested by Gerlofs-Nijland et al. (2005). Because of the important role of macrophages in subchronic inflammation, one would expect their increase in BALF after particulate exposure, but our 24-h time point was probably too early for that effect. This view is supported by the study of Walters et al. (2001) and by the study of Jussila et al. (2001) on bioaerosol particles.

There was no systematic response pattern in BALF total protein or LDH concentration at any of the used time points. Similar findings have been reported by other recent studies (Dick et al., 2003; Gerlofs-Nijland et al., 2005), suggesting generally a low acute cytotoxicity of urban air particulate samples.

The BALF cytokine (TNF, IL-6, KC) responses to both the PM_{10-2.5} and PM_{2.5-0.2} samples followed a clearly faster response pattern than the total cell number or total protein responses. Large increases in concentrations of all the cytokines were consistently measured at 4 h, which was followed by a rapid decline. At 12 h, the cytokine levels (especially KC) remained higher with the PM_{10-2.5} samples than with the PM_{2.5-0.2} samples. In several previous studies (Dick et al., 2003; Gavett et al., 2003; Schins et al., 2003), BALF cytokines have not been sensitive markers of inflammation in rodents exposed to urban air particulate samples. According to our present findings, this may be largely due to a too late time point for the BALF cytokine measurement in acute inflammatory response. However, Gerlofs-Nijland et al. (2005) have reported that coarse road tunnel dust particles induce relatively large increases in TNF-α, IL-6 and MIP-2 concentrations in rat BALF, not only at 4 h after exposure, but the responses remain at high level up to 24 h. Thus, urban air particulate samples induce large increases in BALF cytokines within a few hours after intratracheal exposure, but there may be some species or strain differences in duration of the responses.

Differences in Pulmonary Responses Between the Sampling Campaigns

The relative toxic activities of the single dose of 10 mg/kg of particulate samples were compared between the sampling campaigns by maximal response at any used time point (4, 12, or 24 h) as shown in Table 2. The toxic activities of the PM_{10-2.5} and PM_{2.5-0.2} samples were compared on an equal mass basis and per cubic meter of ambient air in order to build a connection to the real-life particulate pollution situation impacting people’s respiratory tract. This is important, because there were large differences in the size-segregated particulate mass concentrations between the six sampling campaigns due to season-specific meteorological conditions and local emission sources.

There were relatively small differences between sampling campaigns with regard to the activities per unit of mass of the PM_{10-2.5} samples increasing BALF total cell number, total protein or cytokins (Table 2). However, the Mediterranean warm and dry season sampling campaigns had the highest mass concentrations of PM_{10-2.5} and, consequently, the highest overall inflammatory activities per cubic meter of urban air. A bit surprisingly, the Amsterdam PM_{10-2.5} showed the highest activity per unit of mass in increasing BALF total cell number, although sea salt has been estimated to comprise about one third of its mass (Sillanpää et al., 2006).

The PM_{2.5-0.2} samples showed larger heterogeneities in their BALF cytokine (IL-6 and KC) responses than the PM_{10-2.5}

<table>
<thead>
<tr>
<th>Size Range</th>
<th>Campaign</th>
<th>Relative response per μg of PM mass/per m³ of air</th>
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<td>Cell number</td>
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<td>1.6</td>
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<tr>
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<td>1.1</td>
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<tr>
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<td>2.8</td>
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<tr>
<td>Helsinki</td>
<td>1.0</td>
<td>2.1</td>
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<tr>
<td>Barcelona</td>
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<td>5.6</td>
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<tr>
<td>Athens</td>
<td>1.3</td>
<td>6.2</td>
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<tr>
<td>PM_{2.5-0.2}</td>
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Note. In the left column for each studied parameter, the value 1 is given to the group of mice showing the smallest response to an equal mass dose (10 mg/kg) of coarse (PM_{10-2.5}) and fine (PM_{2.5-0.2}) particulate samples. In the right column, a similar comparison of toxic activities is made per cubic meter of urban air. (See Table 1 for particulate mass concentrations).

The largest value for each parameter bolded.
samples. The samples from Athens summer and Barcelona spring campaigns induced rather consistently the highest inflammatory activities in all the measured parameters, which could be associated with their highest atmospheric photochemical activities of all the campaigns (Sillanpää et al., 2005). The samples of Prague winter, Amsterdam winter, and Duisburg autumn had most often much lower inflammatory activities. Especially in the Prague campaign, one possible explanation is the high contribution to fine particulate organics by local biomass and coal combustion as assessed by their tracers, levoglucosan and arsenic, respectively (Sillanpää et al., 2005). Both of these local combustion sources are likely contributors to the high PAH concentrations measured in Prague (Sillanpää et al., 2004), and PAHs have immunosuppressive effects decreasing cytokine production in the rat lung (Kong et al., 1994). The PAH contents of the particulate samples are lowest in our Mediterranean campaigns, which may due to a rapid photo-oxidation of these compounds in warm and sunny atmospheric conditions (WHO IPCS, 1998). Also in studies in vitro (Dybing et al., 2004; Becker et al., 2005), particulate samples collected during warm seasons have had higher inflammatory activities than samples collected in colder seasons.

The comparison of relative toxic activities could not be made for the PM0.2 samples, because the inflammatory responses to these particulate samples were negligible. Only a few previous toxicological studies have investigated inflammatory effects of outdoor air ultrafine particles. In an inhalation study, Kooter et al. (2006) did not add anything to the minor effects of concentrated ambient air fine particles from a Dutch traffic environment in spontaneously hypertensive rats. Moreover, the in vitro studies support our negative findings. Becker et al. (2003, 2005) have reported that collected urban air ultrafine particles have much lower inflammatory activity than the corresponding fine particles. Jalava et al. (2006), using the same particulate sampling technique and sample preparation procedures as the present study, have shown that both the PM0.2 samples and the accumulation size range (PM1.0-2.5) samples have much lower inflammatory activity in a macrophage cell line than larger fine particles (PM2.5-1) and coarse thoracic particles (PM10-2.5). These findings are consistent over different short-term air pollution situations in Helsinki, although only PM0.2 is collected on the glass fiber filter. The PM1.0-2.5 samples are collected on PUF and treated similarly to the PM2.5-1 and PM10-2.5 samples. Thus, the inflammatory activity of urban air ultrafine particles seems lower than that of, for example, industrial titanium dioxide or carbon particles in the same size range (Oberdörster, 2000; Stoeger et al., 2006). This may be due to a higher solubility and different surface properties of the urban air ultrafine particles.

Methodological Considerations

The present study utilized a controlled intratracheal instillation technique for the delivery of size-segregated particulate samples to the lower airways of mice. Of course, this exposure differs from inhalation exposure to actual concentrated ambient air particles (CAP). However, the two exposure techniques have resulted in similar particulate distribution patterns and inflammatory responses in the rodent lungs (Driscoll et al., 2000; Costa et al., 2006). Testing several dose levels and time points would not be possible with CAPs obtained directly from ambient outdoor air, because the aerosol properties change continuously. Nor would a re-aerosolization of previously collected particles be feasible in our study, as it would require much larger amounts of particulate mass than what is available for the instillation technique.

The doses of particulate matter used in our present intratracheal instillation study seem relatively high when compared to lung doses that are likely to be delivered acutely to the lungs in CAP studies. However, there are no higher than the instilled doses that have usually induced inflammatory responses in rodent lungs according to the cited literature (Adamson et al., 1999; Walters et al., 2001; Schins et al., 2004; Gerlofs-Nijland et al., 2005). We emphasize that the use of this kind of doses is required to make it possible to show statistically significant differences in the inflammatory activity between the particulate samples. This is especially true for small groups of healthy animals used in our study. Moreover, not all particulate matter instilled to the trachea reaches the deep lung but part of it is removed via mucociliary clearance like with inhalation. Animals with preexisting chronic lung disease may be generally more sensitive to the inflammatory effects of particulate samples, but the disease status would bring additional variability to the responses. Anyway, we did not observe any signs of lung overloading even with the largest mass dose of 10 mg/kg. Our present study, however, suggests that the use of somewhat lower doses may be possible when the most appropriate time points for each measured parameter are used in response recording.

CONCLUSIONS AND IMPLICATIONS

Our results show the importance of knowing the time course of acute inflammatory response in different parameters used in investigation of the inflammatory activities of ambient air particulate samples in the rodent lung. The maximal responses in BALF cytokine concentrations appear within a few hours and last much less time than those in BALF inflammatory cell number and total protein concentration. This means that more than one time point is needed for a reliable assessment of the cytokine-dependent acute inflammatory response to urban air particles in vivo. With three time points, we managed to show not only strong inflammatory activities but also a great sampling campaign-dependent heterogeneity in response profiles. Thus, particulate size, sources, and atmospheric transformation processes affect the inflammatory activity and response duration of urban air particulate matter in the mouse lung. These experimental findings may help explaining some of the observed heterogeneities in particulate exposure-response relationships with human adverse health outcomes in epidemiological studies.
REFERENCES


