

Respiratory allergy adjuvant and inflammatory effects of urban ambient particles

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Abstract

PM₁₀ and PM_{2.5} fractions were collected by high-volume cascade impactors during 4-week periods in spring, summer and winter seasons in Amsterdam, Lodz, Oslo and Rome and at a Dutch seaside site. The samples were screened for respiratory allergy potential with the mouse popliteal lymph node (PLN) and the ELISA-based IgE antibody assays. For inflammatory screening, release of the cytokine macrophage inflammatory protein-2 (MIP-2) from primary rat type 2 cells was determined. Most fractions gave an increase in the lymph node response with the model allergen ovalbumin indicating adjuvant activity. Some of the coarse fractions gave a lymph node response even in the absence of ovalbumin, caused probably by non-specific inflammation. With the exception of a few of the coarse fractions, all ambient fractions increased the production of specific IgE. Fine particles had stronger adjuvant effect than coarse particles. A significant increase in the allergen specific IgG2a response was observed for the fine and some of the coarse fractions, indicating a non-allergic Th1 response. No consistent differences in adjuvant effects between the locations were observed. Particle samples collected in the different European cities differed in their potency to induce MIP-2 in type 2 cells. Coarse fractions of the urban particles samples, as well as the coarse fraction collected at the seaside, were more potent than the fine fractions to induce MIP-2. With respect to seasonal variations, the coarse fractions collected in summer seemed to be the most potent.

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1. Introduction

Inhaled ambient particles have been found to be associated with adverse short-term health effects, such as increased cardiopulmonary mortality in elderly individuals and the exacerbation of asthma in all age

groups (de Hartog et al., 2003; Pope et al., 1999, 2002; von Klot et al., 2002). In the APHEA-study correlating exposure to urban air pollutants among 12 European cities with short-term health outcomes, regional as well as seasonal differences in the estimated pooled risks were observed (Katsouyanni et al., 1997). With regard to long-term health effects, in particular the development of allergy and asthma, evidence for an adverse effect from particulate exposure is less obvious, but some epidemiological studies have reported an association between the prevalence of asthma and

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allergy or pulmonary function and road traffic pollution (Janssen et al., 2003). Ecological epidemiological studies have demonstrated marked differences in the prevalence of allergy and/or asthma between former west and east Germany (von Mutius et al., 1992, 1994) and between Hong Kong and other regions of China (Leung and Ho, 1994, 1997; Diaz-Sanches et al., 1999). Self-reported asthma is about eight times higher in the UK than in south-eastern countries such as Greece, Georgia and Romania (ISAAC, 1998).

Given that there are widely different prevalence rates of respiratory allergies and asthma between the countries of Europe and exposure to ambient particles is substantial in urban environments throughout Europe, the following research question was formulated: May qualitative differences in air pollution at different locations in part explain differences in prevalence or severity of respiratory allergies throughout Europe? To answer this question, a research project named “Respiratory Allergy and Inflammation Due to Ambient Particles—a European-wide Assessment (RAIAP)” was initiated in 2001. PM₁₀ and PM_{2.5} ambient particulate fractions were collected in the cities of Amsterdam (The Netherlands), Lodz (Poland), Oslo (Norway) and Rome (Italy), and at a Dutch seaside background site during the spring (pollen), summer and winter seasons. The particles have been characterised for physical, chemical and immunological parameters. In the present communication, part of the results from the screening studies of respiratory allergic and inflammatory potential of the collected RAIAP samples will be described.

2. Methods

PM fractions were collected on polyurethane foams with the aid of two high-volume (1.100 l/min) cascade impactors with a multi-stage round slit nozzle impactor (Kavouras et al., 2000). The sampling campaigns were performed close to busy streets in the four cities during staggered 4-week periods from March 2001 until March 2004. Particulate samples were extracted with methanol from the polyurethane foams, evaporated and dried. During the same periods, low-volume (1–2 l/min) sampling on carbon coated and germanium coated polycarbonate filters was also conducted for analytical transmission

electron microscopic studies. The following measurements were performed on the high-volume samples: mass and concentration, 32 elements, 5 inorganics, 23 PAHs, 7 hopanes and stearanes (traffic markers) and endotoxins (Cassee et al., 2003).

For assaying respiratory allergy potential, the popliteal lymph node (PLN) assay (Descotes and Verdier, 1995; Lovik et al., 1997) and an ELISA-based IgE, IgG1 and IgG2a ovalbumin-specific antibody assays (modified after Ormstad et al., 2003) were used. In the PLN assay, Balb/cA mice were injected in one hind foot pad with ovalbumin (50 µg), with or without particles (100 µg), or particles alone on day 0. The draining popliteal lymph nodes were harvested on day 6 and weight. Cell number and ex vivo in vitro proliferative indices (³H-thymidine incorporation, without in vitro stimulation) were determined. Diesel exhaust particles (DEP, 50 µg) (1650, US National Institute of Standards and Technology) were included as a positive reference particle. In the specific antibody assays, serum levels of ovalbumin-specific IgE, IgG1 and IgG2a were determined on day 26 after similar inoculation (100–200 µg particles), 5 days after a second injection with ovalbumin without particles on day 21.

For inflammatory screening, human lung alveolar epithelial cells (A549), primary human small airway epithelial cells, primary rat type 2 and Clara cells, and rat alveolar macrophages were used. With respect to experiments with primary rat type 2 cells, which are presented here, the cells were exposed to various concentrations of particle samples for 20 h with addition of foetal bovine serum 6 h after the start of exposure. The culture medium was analysed for the cytokine macrophage inflammatory protein-2 (MIP-2) using a commercial ELISA kit (Biosource International, Camarillo, CA, USA).

3. Results

3.1. Allergy screening

To be able to compare the different sampling sites, the coarse and fine fractions were examined in separate PLN assay experiments. In Fig. 1A and B the popliteal response to ambient particulate matter collected in Oslo, Rome, Lodz, and Amsterdam in the spring season is shown (data from summer and winter

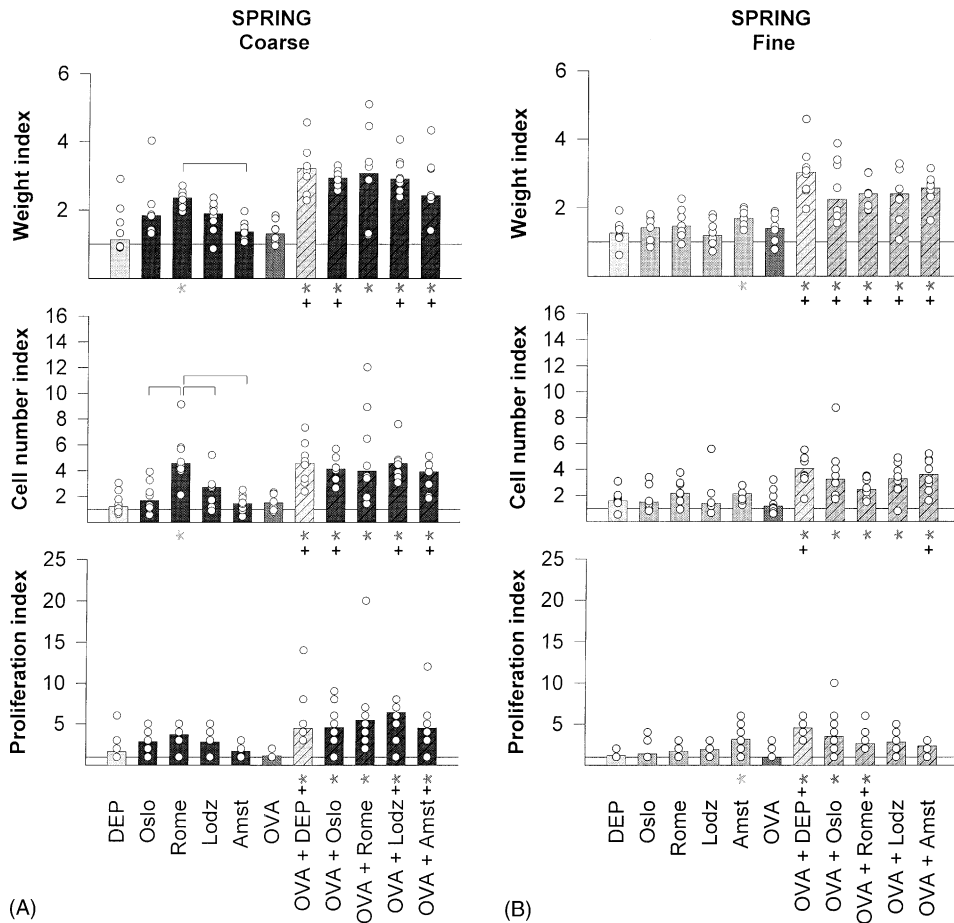


Fig. 1. Weight (upper panels), cell number (middle panels), and proliferative indices (lower panels) in the popliteal lymph node 6 days after footpad inoculation of Balb/cA mice as specified in Methods for coarse (A) and fine (B) ambient particles collected in the spring season. An index above 1 signifies activity. Individual (points) and medians (columns) for groups of eight mice are shown. The following comparisons were made all “particle alone” groups vs. diesel exhaust particles (DEP) (*). All “particle and ovalbumin (OVA) groups” vs. “OVA” (*), and vs. the respective “particle alone” group (+). Brackets denote statistically significant differences between similar preparations with particles from different sites.

samples not shown). No, or only weak weight, cellular, and proliferative responses to ovalbumin (OVA) given without particles was obtained. In the presence of particles, however, the OVA response showed a statistically significant increase (indicated by stars), indicative of an adjuvant effect. Particles elicited generally only weak popliteal responses in the absence of OVA. Following a pairwise comparison of particles from each location, a significantly stronger weight and cell number response in the presence than in the absence of OVA was observed for a majority of the fractions (indicated by plus signs). An exception was

the coarse summer samples (not shown), as well as the spring sample from Rome, which seemed to be more irritating than the remaining ambient fractions.

To further examine the adjuvant activity of the ambient particulate matter, its ability to elicit an allergen specific IgE and IgG1 response was analysed, indicating an allergy-related Th2-immune response. The results obtained are presented in Fig. 2 for ambient particles collected in the spring. The antibody response to OVA was usually significantly stronger as compared to the control groups. Moreover, all the European ambient particle fractions, from all seasons, significantly

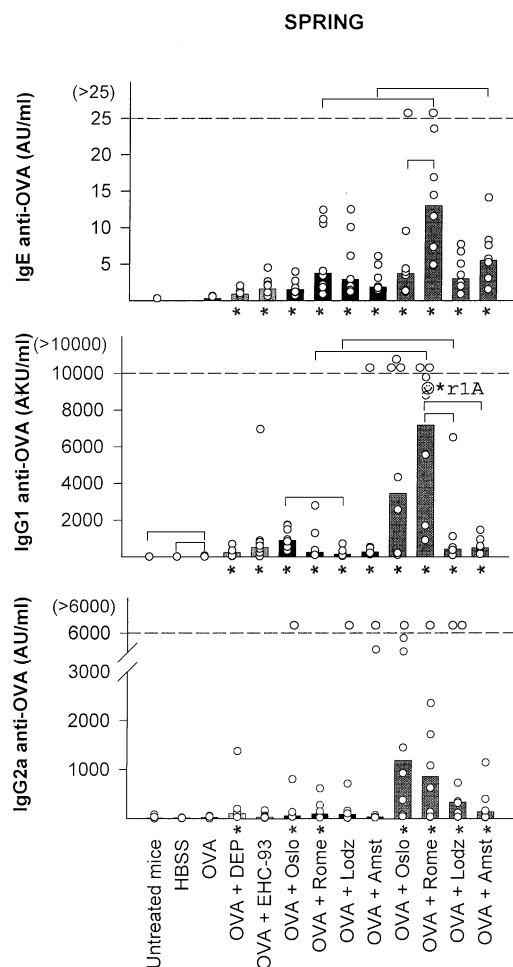


Fig. 2. The upper, middle and lower panels show the levels of IgE, IgG1, and IgG2a anti-OVA, respectively, in Balb/cA mice 5 days after footpad inoculation with ovalbumin (OVA), and 25 days after inoculation into the same footpad as specified in Section 2. EHC-93 is a reference sample of urban air particles collected in Ottawa. Dark columns represent the coarse fraction and light columns the fine fraction, respectively. Individual (points) and medians (columns) for groups of eight mice are shown. Asterisks indicate a statistically significant stronger response compared to OVA. Brackets denote statistically significant differences between similar preparations with particles from different cities, and between coarse and fine fractions from the same location.

increased the Th1-dependent IgG1 response to OVA. With the exception of a few coarse ones, all fractions also significantly increased the IgE anti-OVA response. All fine fractions, and some of the coarse ones, gave a significant increase in the IgG2a response to

OVA as well. Thus, it seemed as the ambient particles not only are able to stimulate an allergic Th2-, but also a non-allergic Th1-response. Although not observed for all locations, it seemed as the fine particles have a stronger adjuvant activity than the coarse ones following a pairwise comparison of the coarse and fine particles from the same location. Taken together, only a few marked differences in the antibody response were observed between the locations.

3.2. Inflammation screening

When type 2 cells were exposed to the various PM samples, different levels of MIP-2 release were induced (Fig. 3A and B). As had been seen with macrophages (data not shown), the coarse fractions were more potent than the fine fraction. With respect to the coarse fractions collected in the pollen (spring) season, the Lodz and Rome samples induced higher levels of MIP-2 than the samples from Oslo and Amsterdam. Among the summer samples, the coarse fraction from Lodz demonstrated the highest potency, whereas no marked differences in potency were observed between the coarse fractions from Rome, Oslo and Amsterdam. The observed decrease in MIP-2 at exposure above 25 $\mu\text{g}/\text{ml}$ of the coarse particles from Amsterdam may be explained by a decrease in cell survival. Regarding the winter samples, coarse fractions from Lodz, Oslo and Amsterdam were less potent compared to the respective pollen and summer samples. The most obvious differences in seasonal variation in potency to induce MIP-2 were observed within the samples from Lodz, whereas no marked seasonal variation was revealed between the coarse fractions from Rome. As compared to the macrophages, the coarse fraction from the seaside elicited a marked increase in cytokine release in the type 2 cells. The potency was at the same level as the coarse fractions collected in Rome. Only minor responses were observed by the fine fractions from the urban sites.

4. Discussion

The allergy screening for adjuvant activity show are that ambient particles, with the exception of a few of the coarse fractions, collected in the four cities of Europe: Oslo, Rome, Lodz and Amsterdam, in the three

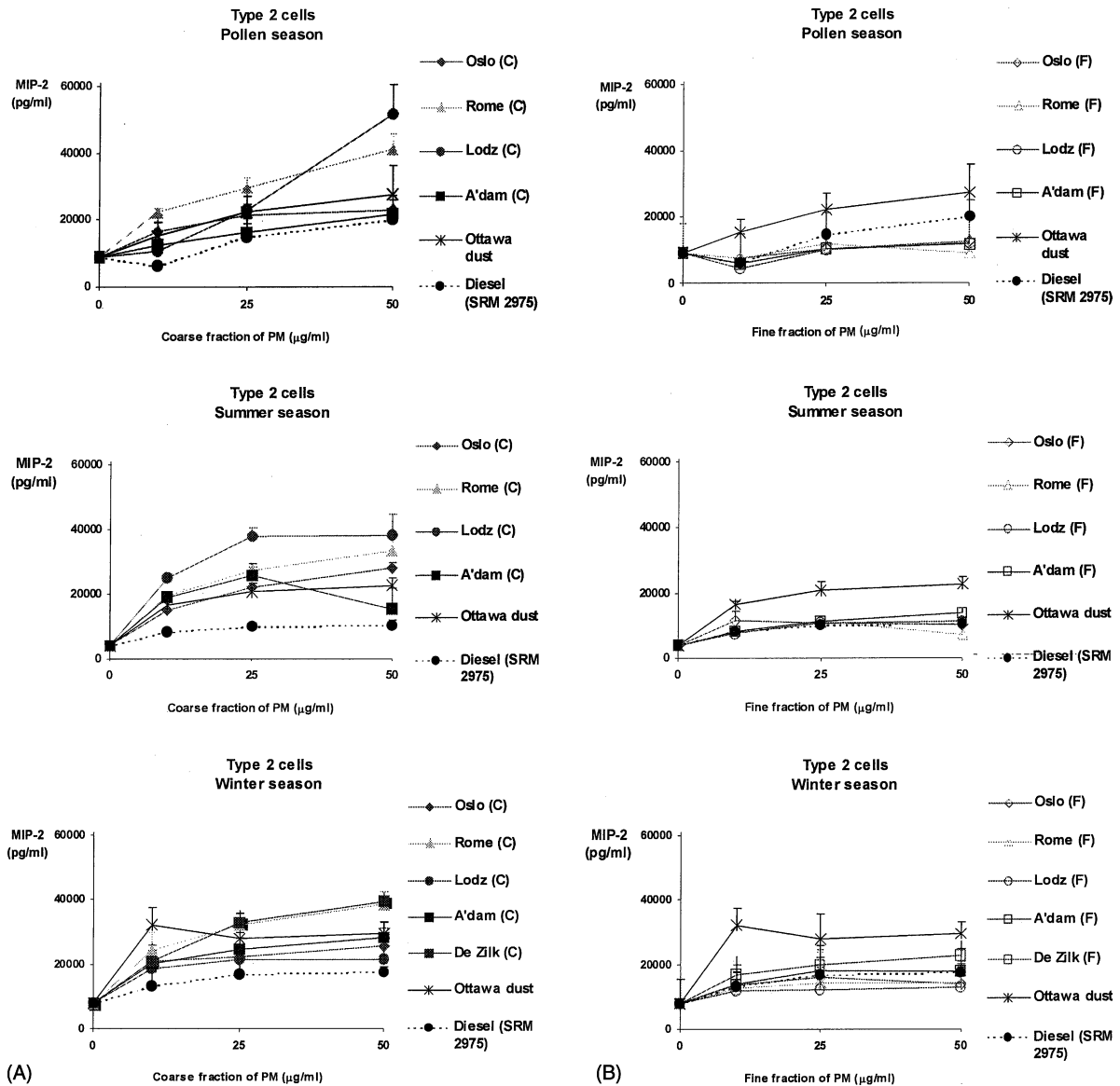


Fig. 3. Release of MIP-2 from type 2 cells exposed to coarse (A) or fine fraction (B) of ambient PM collected in different European cities during pollen (upper), summer (middle) and winter (lower). Results are mean $\pm$ S.E.M., $n = 3$.

seasons (spring, summer, and winter), have an adjuvant activity in the doses examined (100–200 μg per mouse). It appears, however, as the fine fractions had a stronger adjuvant activity than the coarse ones. Few marked differences were observed between locations. We cannot exclude, however, that the popliteal lymph node assay and the allergen specific ELISA-based

IgE assay, with the immunisation protocol used, were not sensitive enough to differentiate between smaller differences in the adjuvant capacity of ambient particulate matter. Alternatively, ambient particles from different locations may not differ substantially in qualitative characteristics important for allergy adjuvant activity. Conceivably, differences in qualitative

characteristics of particles may not influence the allergy adjuvant effect.

Our results are in agreement with those obtained by van Zijverden and Granum (2000) and Granum et al. (2001), who found particles generally seem to have an adjuvant activity. However, different particles appear to stimulate distinct immune responses to an allergen. Whereas DEP seem to skew the immune response in a Th2-direction, silica particles skew it in a Th1-direction (van Zijverden and Granum, 2000). In the present study, DEP was mainly found to elicit a Th2-immune response. The ambient particulate matter samples, on the other hand, which are composed of different types of particles including both combustion and silica particles, elicit a Th2- as well as a Th1-immune response.

The results obtained using the relatively simple 6 days popliteal lymph node assay seem to correlate well with the results obtained using the more sophisticated 26 days ELISA-based allergen specific IgE assay. Both assays, in which mice are sensitised by footpad inoculation, appear to be suitable as allergen screening assays. The popliteal lymph node assay may be further simplified by leaving out measurements of proliferative responses, which are time-consuming and costly considering the information obtained. However, the popliteal lymph node assay does not distinguish between an allergic and non-allergic immune or inflammatory response. Further, IgE represents a biologically relevant endpoint. In the present experiments, the relatively strong IgE adjuvant activity of fine particulate samples from Rome (spring) and Lodz (summer, data not shown) was not reflected by the popliteal lymph node response. The IgE assay may therefore be both more specific for allergy adjuvant activity and be more sensitive. It further remains, however, to compare the IgE responses measured following footpad inoculation with those obtained following a more real-life exposure such as intranasal administration.

The results from the present study of inflammatory responses in primary rat type 2 cells demonstrated that ambient air particles collected at different sites may differ in their potency to induce inflammatory markers. Ambient air particles are a complex mixture of particles with different sources in urban areas. The observed variations may therefore be due to differences in physical or chemical characteristics of the particle samples collected in the indicated cities

during three seasons. In addition to possible size distribution and surface area variations, metal content might play an important role for potency differences. Thus, the importance of metals for eliciting the release of pro-inflammatory cytokines from lung cells and in whole lung has been elucidated in several studies (Carter et al., 1997; Ghio et al., 2002; Molinelli et al., 2002). Transition metals such as Fe and Cu have been shown to participate in the production of reactive oxygen species (ROS) (Ghio et al., 1999). Recent studies have demonstrated that the non-redox metal Zn also can be involved in lung injury and inflammation (Prieditis and Adamson, 2002; Kodavanti et al., 2002). Furthermore, components that might be bound to the particles, such as endotoxins and other organic compounds, could contribute as inducers of biological responses. High organic carbon and polycyclic aromatic hydrocarbon (PAH) content of particles has been correlated with oxidative stress induced by ultra-fine PM in macrophages and epithelial cells (Li et al., 2003). However, correlation studies have not yet been finalised.

In the present study, the coarse fractions were more potent to induce MIP-2 than the fine fractions. In support of these findings, the coarse fraction of PM₁₀ induced cytokines to a much higher extent than the PM_{2.5} fraction in alveolar macrophages (Soukup and Becker, 2001). This cytokine release was partly inhibited by polymyxin B and LPS-binding protein, implicating endotoxin as a cytokine-inducing moiety. Thus, the higher potency of the coarse fractions in our study may be due to particle-bound endotoxin.

The results from the type 2 cell experiments indicated that coarse particles collected during summer were the most potent. This was most evident with the coarse particles from Lodz, whereas seasonal variation was less obvious with the particles from Rome. Whether the seasonal variations may be due to chemical or physical differences remains to be clarified. Shi et al. (2003) found that weekly collected samples in two different townships varied in their potential to induce ROS formation from week to week. Also this result might indicate variations in the particle properties that change relatively frequently.

In this study only the results with rat lung cells have been reported. To achieve a broader database on which to judge the *in vitro* effects of particles, experiments need to be performed in different types of

lung cells from both rats and humans. Previous studies have shown that different cell types may give different responses (Hetland et al., 2003). The in vitro results can then be verified in limited in vivo experiments. In a study with mineral particles the results of cytokine release in vitro and neutrophil influx in vivo correlated well (Becher et al., 2001). The results of these extended in vitro and limited in vivo studies are currently being analysed.

Taken together, the results from the present in vitro screening of inflammatory responses in different types of lung cells demonstrate that ambient air particles collected at different sites may differ in their potency to induce inflammatory markers. The observed variations may be due to differences in physical or chemical characteristics of the particles. Furthermore, components that might be bound to the particles, such as endotoxins and other organic compounds, may contribute as inducers of biological responses. The results from the present studies, where different types of lung cells were exposed to particles of different sampling sites, showed that the order of potency among the particle samples was relatively similar from one cell type to another (here only results from the type 2 cells are shown). Among the coarse PM collected during pollen and summer seasons, the overall result was that the coarse fractions from Lodz and Rome were more potent than the coarse fractions from Oslo and Amsterdam. Furthermore, the results indicated that coarse particles collected during winter were less potent than coarse particles collected during the pollen (spring) and summer season. This was most evident with the coarse particles from Lodz, whereas seasonal variation was less obvious with the particles from Rome.

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