Particulate matter in the environment: pulmonary and cardiovascular effects

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Purpose of review

The mechanisms related to adverse respiratory and cardiovascular effects in populations exposed to particulate matter are under debate and different models have been used to further our understanding of the various aspects of those effects. In this review we present some studies that may give new insights into the cellular and systemic mechanisms related to particulate matter toxicity.

Recent findings

Strong epidemiological evidence is now available regarding exposure markers and health effects. This is evident in the correlation between carbon content in macrophages and decrease in lung function, an increase in the risk of chronic obstructive pulmonary disease, lung cancer and postnatal mortality. The role of outdoor temperature and a missing allele for *GSTM1* and the impact of these factors on cardiovascular effects are also reported. At the experimental level, the effects of particulate matter and the interactions between different cell types, the role of toll-like receptor-2 and 4, the translocation of particles through cell monolayers and the activation of endothelial cells by particulate matter are also discussed. The role of composition is under intense debate, and different statistical analyses have been proposed.

Summary

Experimental studies on the effects of particulate matter are giving plausibility to the epidemiological findings, but the possible mechanisms of action are also becoming a hot topic.

Keywords

cardiovascular effects, PM_{10}, $PM_{2.5}$, pulmonary effects, ultrafine particles

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Abbreviations

COPD	chronic obstructive pulmonary disease
DEP	diesel exhaust particle
TLR	toll-like receptor
UFP	ultrafine particle

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Introduction

Airborne particulate matter has been associated with increases in morbidity and mortality. The historical events of the Meuse valley fog of 1930 [1] and the London smog of 1952 [2] triggered successful efforts and legislations to reduce air pollution. Nevertheless, a relation between urban air pollution and morbidity and mortality persists even at present levels of urban pollution [3]. Understanding the mechanisms of the adverse health effects caused by particulate matter represents an important scientific challenge. One of these challenges is to explain which properties of particulate matter are critical in causing injury. Particles capable of penetrating into the lower respiratory tract, and even into the alveolar region, are those with a mean aerodynamic size of up to 10 μm (PM_{10}), up to 2.5 μm ($PM_{2.5}$) and up to 0.1 μm (ultrafine particles, UFPs) [4]. Particulate matter can trigger multiple cellular responses in the lung such as cytotoxicity, inflammation, and mutagenesis. Moreover, the adverse effects of particulate matter are not confined to the lung, but there are also extrapulmonary manifestations of exposure to particulate matter. These systemic effects may be due to a 'spill-over effect' of pulmonary inflammation or to translocation of a fraction of particles into the circulation.

We review the recent advances in the epidemiologic and mechanistic research on particulate matter pollution, with some bias towards our own contributions to the field. An excellent and extensive general review on the topic of air pollution has been recently published by Pope and Dockery [5••].

Respiratory effects of particulate matter

Exposure to particulate matter may have different outcomes, including lung function deterioration, chronic obstructive lung disease, allergic illness and lung cancer (Fig. 1).

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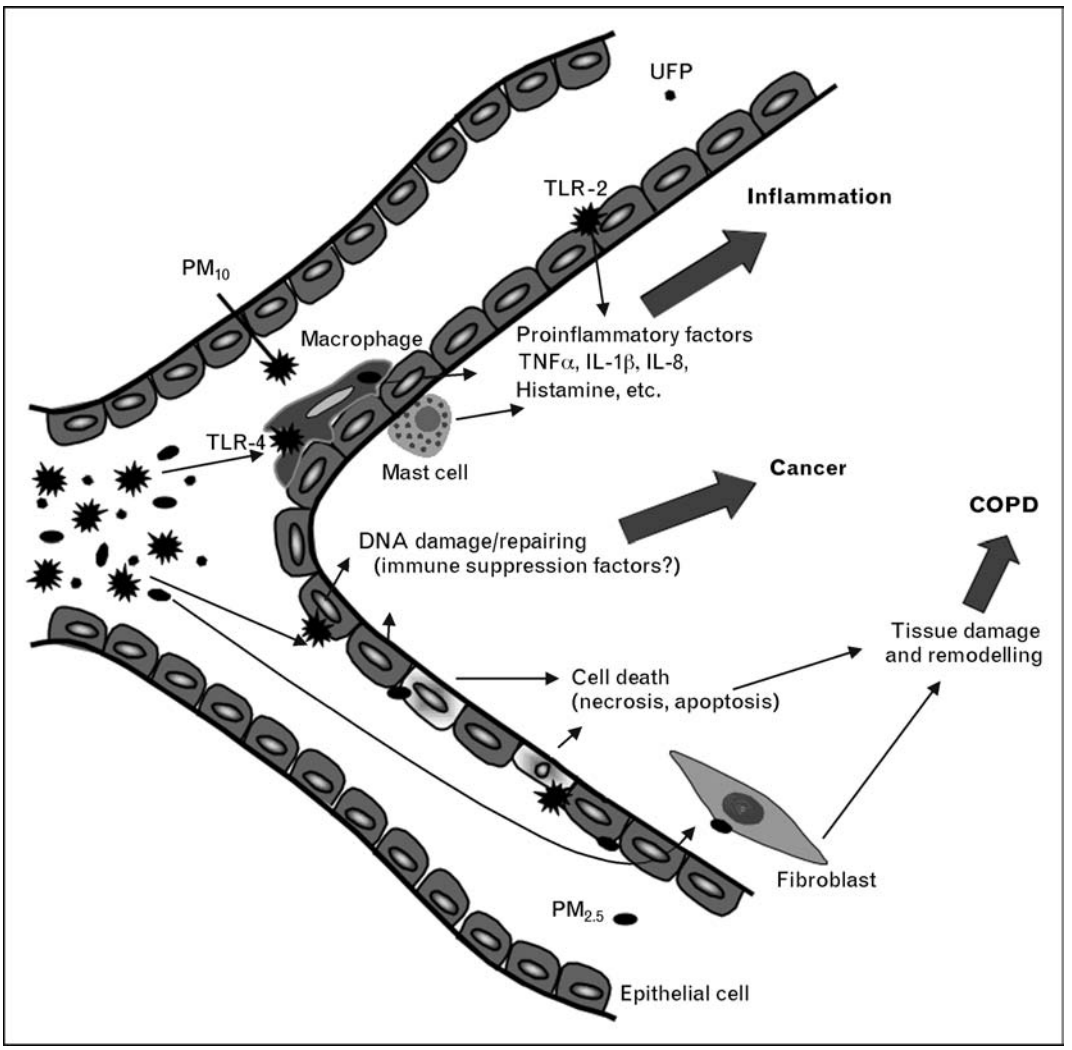
Figure 1 Diagram of the possible targets and effects of particulate matter (PM) on the airways



Cell death (necrosis, apoptosis), proinflammatory factors secretion, DNA damage, tissue damage and remodelling. COPD, chronic obstructive pulmonary disease; IL, interleukin; TLR, toll-like receptor; TNF, tumor necrosis factor; UFP, ultrafine particle.

Pre and postnatal effects of air pollution

Several studies have shown adverse effects of particulate matter on pregnancy outcomes, including preterm delivery, lower birth weight and an increase in postneonatal infant mortality. A PM_{10} increase of $10\,\mu g/m^3$ is associated with a 5% increase in postneonatal mortality for all causes and around 22% for postneonatal mortality from respiratory diseases [6•]. The critical periods and the mechanisms remain unclear.

Lung function

Exposure to air pollution during childhood may impair the development of the lung and thus lead to a lower level of peak pulmonary function in early adulthood. Kulkarni *et al.* [7••] were able to show, in children, that the load of carbon particles found in airway macrophages, recovered from induced sputum, correlated with their exposure to particulate matter and that this biologic index of exposure was inversely correlated with lung function. Thus, each $1.0\,\mu g/m^3$ increase in primary PM_{10} was associated with an increase of $0.10\,\mu m^2$ in the carbon content of airway macrophages, and each increase of $1.0\,\mu m^2$ in carbon content was associated with a reduction of 17% in forced expiratory volume in 1 s, of 12.9% in forced vital capacity, and of 34.7% in the forced expiratory flow between 25 and 75% of the forced vital capacity. These results strengthen the observations from Gauderman *et al.* [8] who reported that increased exposure to PM_{10} was associated with impaired growth of lung function.

Allergic illness

Animal studies, as well as controlled human exposure studies, have shown that diesel exhaust particles (DEPs)

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can increase the risk of allergy development and exacerbate allergic reactions [9,10]. The evidence from population studies to support this observation is still conflicting, possibly as a result of differences in the mix of transport-related air pollutants [11].

Chronic obstructive lung disease

Acute exacerbations of chronic obstructive pulmonary disease (COPD) have been associated with short-term variations in air pollution [12]. Schikowski *et al.* [13••] investigated the influence of long-term exposure to air pollution on respiratory symptoms and pulmonary function in middle-aged women. A 7 $\mu g/m^3$ increase in 5-year means of PM_{10} (interquartile range) was associated with a 33% increase in the risk of COPD. These findings were confirmed when replacing the estimates of PM_{10} by distance of home to a major road.

Lung cancer

Until now, six prospective studies have indicated that long-term exposure to urban pollution is likely to increase the risk of lung cancer [14,15•,16–19]. Compared with the risk associated with active smoking, the risk associated with air pollution is low (+10–20% for each 10 $\mu g/m^3$ increase in PM_{10} or $PM_{2.5}$). A recent European cohort study (European Prospective Investigation into Cancer and Nutrition) yielded a positive but nonsignificant association between lung cancer incidence and residence near heavy traffic roads (1.46, CI 0.89–2.40) [14]. In the same cohort, however, a significant interaction was found between the exposure variable 'distance from heavy traffic road' and polymorphisms in genes coding for base excision repair (*XRCC1-Arg399Gln*) and double-strand break repair (*BRCA2-Asn372His*) [20••]. An ecological study suggests that some of the differences in lung cancer mortality within Europe could be explained by exposure to particulate matter pollution [21].

Experimental studies

Various outcomes such as cell necrosis and apoptosis [22–26], secretion of cytokines (tumor necrosis factor α (TNFα), interleukin (IL)-1β, IL-6, IL-8) [22,25–27] and DNA damage (mutation assays, single and double-strand breaks, DNA degradation, and comet assay) [25,28,29] have been described upon incubation of various types of cells with particulate matter. Two main questions remain: what are the mechanisms involved and which components of the particulate matter are responsible for the effects?

Rats instilled with PM_{10} present a mild oedematous response 3 days after exposure [30•]. This response was linked to an increase in the expression of genes for the ion channel-acetylcholine receptor, IL-2 and serine/threonine kinase. The lack of other markers was explained by an earlier response that may not last long. In-vitro evaluation of particulate matter on epithelial cells, macrophages and lymphocytes provided new evidence in this direction: proinflammatory mediators such as TNFα, IL-1β, IL-8 are secreted in parallel to antiinflammatory mediators such as IL-6 and transforming growth factor β (TGFβ) [31•], and this response is constant up to 72 h of exposure. In another study, UFPs showed cytoskeletal dysfunction in macrophages [32•]. The balance between proinflammatory/antiinflammatory effects and an inefficient clearance of particles (due to dysfunction of macrophages) could lead to chronic oedema.

The upregulation of IL-4 and interferon γ (IFNγ), and the expression of CD69 on T cells exposed to particulate matter could explain exacerbations of asthma [33•]. In a recent study, epithelial cells exposed to particulate matter in the presence of a supernatant of previously exposed alveolar macrophages to the same particles led to a stronger upregulation of several proinflammatory factors [RANTES, TNFα, intercellular adhesion molecule-1 (ICAM-1), IL-1β, membrane cofactor protein, leukemia inhibitory factor and vascular endothelial growth factor] and an enhanced activity of the nuclear factor κB when compared with the response to particulate matter alone [34] correlating with TNFα and IL-1β. Later on, the same group [35••] reported that cocultures of alveolar macrophages with epithelial cells have a wide response to particulate matter presenting an upregulation in the expression of macrophage inflammatory protein 1β (MIP-1β), granulocyte–macrophage colony stimulating factor (GM-CSF) and IL-6 after 2 h and in the secretion of MIP-1β, GM-CSF, M-CSF and IL-6 after 24 h of exposure. Interestingly, the interaction between alveolar macrophages and epithelial cells seems to be independent of cross-linking between these two cells.

Recently, toll-like receptor-2 (TLR-2) and TLR-4 have been identified as candidates of how cells interact with particulate matter [36••]. According to this report, the secretion of IL-8 by airway epithelial cells can be inhibited when an antibody against TLR-2 is added, but no effect is observed on alveolar macrophages using the same antibody. In contrast, the response of macrophages to particulate matter seems to be related to TLR-4 but is not affected by the inhibition of TLR-2. These findings are important considering that biological components of the particles have been previously related to their effects [27,37].

Composition of particulate matter and its relation to biologic effects

During the 1990s the toxicity of particulate matter was mostly related to size, and variations in composition were considered less important. Nevertheless, many publications have indicated that composition plays a

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very important role on particle toxicity [25–27], and the observed differences were empirically related to endotoxin, metals and organic compounds. Differences in composition related to particulate size, city and season have recently been reported [38•]. The variations in composition have more impact on the effects (TNFα and IL-6 release) induced by the coarse fraction than the differences in surface area. Independently, a study of different soil-derived particulate matter also presented a high correlation between differences in composition (organic carbon and elemental carbon content) and the secretion of IL-6 and IL-8 in BEAS-2B cells [39••].

Different statistical approaches may be used to evaluate the importance of independent components of particulate matter. The use of multiple regression analysis followed by a stepwise analysis showed that PM_{10} from different regions of Mexico City are mainly related to nitrogen with regard to cytotoxicity, and to lead with regard to proinflammatory effects (E. Alfaro-Moreno, S. Ponce-de-León-Rosales, A.R. Osornio-Vargas, *et al.*, in preparation). In another study using principal component analysis and factorial analysis, independent combinations of components were found to correlate with viability (S/K/Ca/Ti/Mn/Fe/Zn/Pb), TNFα and IL-6 secretion (S/K/Ca/Ti/Mn/Fe/Zn/Pb and endotoxins/organic carbon/black carbon), and p53 expression (high levels of endotoxins/organic carbon/black carbon and low levels of S/K/Ca/Ti/Mn/Fe/Zn/Pb) [40]. In a study evaluating the secretion of cytokines and reactive oxygen species (ROS) induced by particulate matter collected in different months [41••], correlations of IL-6 with Fe and Si were observed, while IL-8 correlated with Cr. In a comprehensive evaluation of the effects of engine emissions (134 components) on 15 different biological outcomes, it was found that re-grouping components by chemical class or subclass allowed for the correlation of lung toxicity with organic carbon, hopanes and steranes; mutagenicity correlated with particle-bound nitrated polycyclic aromatic hydrocarbons [42].

In relation to the genotoxic effects of particulate matter as a possible factor for developing lung cancer (see above), ROS play an important role in DNA damage [43•]. Oxidative DNA damage evaluated on circulating mononuclear cells of healthy nonsmokers was related to outdoor–indoor exposure to UFPs, but no correlation was found with other gaseous pollutants, even considering that correlations among them exist [44••]. An in-vitro study shows that the organic fraction and the washed fraction are less potent inducers of DNA damage than the total particles [45••]. Mutagenic compounds, mainly associated with particulate matter from urban or rural regions (related to a chemical plant) were demonstrated using the Ames test [46•].

Cardiovascular effects of particulate matter

Epidemiologists have linked air pollution with acute and chronic cardiovascular endpoints [47•]. The plausibility of these observations, however, was questionable because little or no experimental studies provided evidence for effects of particulates outside the lungs (Fig. 2).

Acute cardiovascular effects

Dozens of studies have investigated the acute effects of peaks of air pollution [3]. Initially these studies used time-series analyses relating day-to-day variations in atmospheric pollutants with daily variations in deaths or disease. Weather-related differences over the seasons, however, may modify or even confound the association between air pollution and mortality, because both high and low temperatures increase mortality, and temperature is closely associated with pollution. Due to these complex relations, simple statistical adjustments may be inadequate and some effects of pollutants may be associated primarily with outdoor temperature. A recent analysis [48] of Belgian mortality data, stratified across seasons and across quintiles of outdoor temperature, revealed that the short-term effect of PM_{10} on mortality strongly depends on season or outdoor temperature, even in a temperate climate. Thus, the percentage increases in total mortality on days in the highest season-specific PM_{10} quartile versus the lowest season-specific PM_{10} quartile were 7.8% in summer, 6.3% in spring, 2.2% in autumn and 1.4% in winter. This pattern was also observed for cardiovascular mortality. The component-specific toxicity of PM_{10} may differ across the temperature range as suggested in an in-vitro study [38•] in which PM_{10} samples collected in the summer were more potent to induce inflammatory cytokines (IL-6 and TNFα) than samples collected in winter.

At the level of the population, acute effects of air pollution on cardiovascular endpoints have been noted to be significant from middle age on. The highest risk appears to be in people with preexisting cardiac disease [49]. Drugs that modify oxidant defences may influence the susceptibility to particle-induced inflammatory or pro-oxidative responses. In addition to their cholesterol-lowering effect, statins also have potent antiinflammatory properties. Schwartz and colleagues [50••] found that the effect of PM_{10} on heart rate variability was confined to persons missing the allele for *GSTM1* (lower oxidative stress defence), but the association was only apparent in those who were not under statin treatment.

Chronic cardiovascular effects

In the Harvard Six Cities study [14] the adjusted overall mortality rate for the most polluted city versus the least polluted city was 1.26. Cardiovascular deaths accounted for the largest single category of the increased mortality. Among air pollutants, elevation of $PM_{2.5}$ was most

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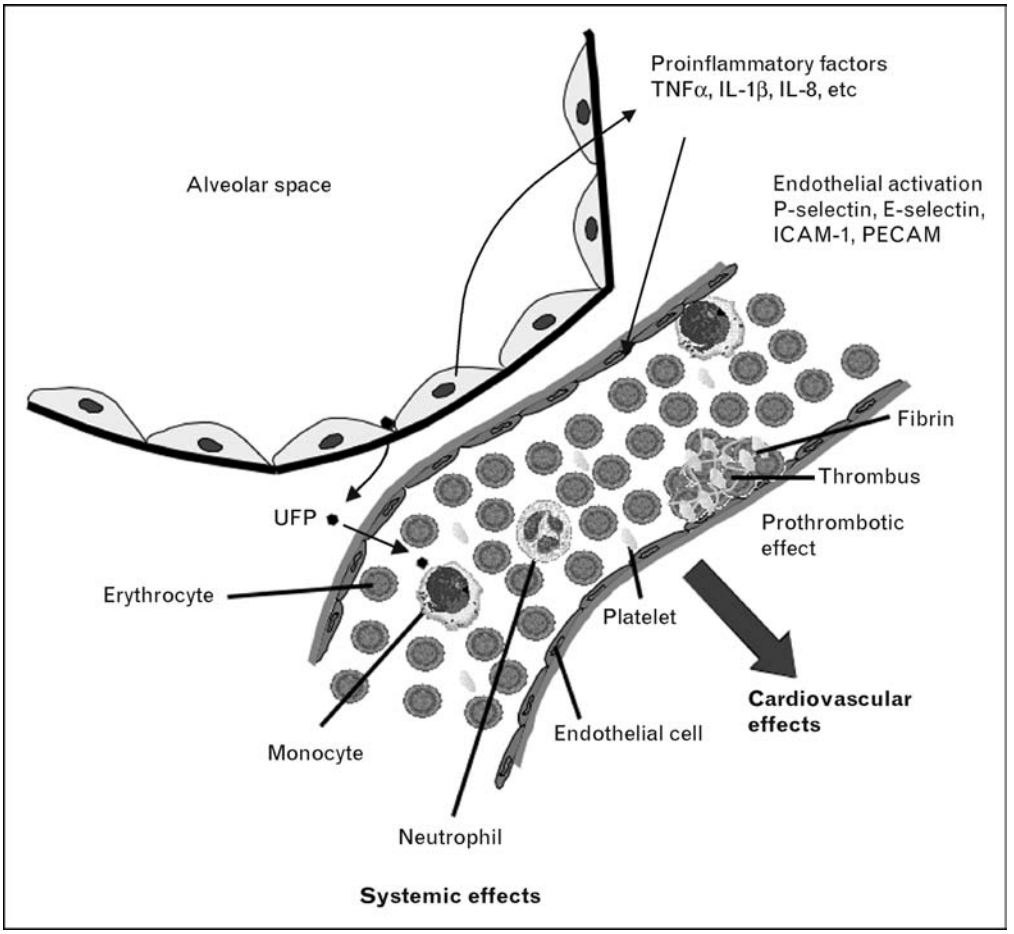
Figure 2 Diagram of the possible mechanisms related to systemic effects induced by ultrafine particles (UFPs)



Proinflamatory factors secretion, particle translocation, activation of endothelial cells and prothrombotic effects. ICAM, intercellular adhesion molecule; IL, interleukin; PECAM, platelet–endothelial adhesion molecule; TNF, tumor necrosis factor.

strongly associated with mortality. Pope *et al.* [51] linked mortality statistics over a 16-year period to chronic exposure to multiple air pollutants in approximately 500 000 adults who resided in all 50 states of the USA. Mean annual $PM_{2.5}$ concentration linearly enhanced cardiovascular mortality, with a significant increase in the risk of death from ischaemic heart disease of 1.18 for an increase in $PM_{2.5}$ of $10\,\mu g/m^3$. The risks for arrhythmia, heart failure, or cardiac arrest mortality were also increased [relative risk (RR) 1.13]. Intriguingly (and hardly discussed in that article), air pollution was associated with a significantly lower risk of dying from COPD (RR 0.84), suggesting either a wrong coding of the cause of death in COPD patients or that patients with COPD are more susceptible to die from cardiovascular complications than from COPD when they have been exposed to high pollution.

Extrapulmonary translocation of ultrafine particles

The impact of inhaled particles on the autonomic nervous system leading to changes in the pattern of breathing, heart rate and heart rate variability is under investigation. Elder *et al.* [52••] demonstrated that the olfactory neuronal pathway represents a significant exposure route of central nervous system (CNS) tissue to inhaled UFPs. These authors showed that, in rats, which are obligatory nose breathers, translocation of inhaled nanosized particles along neurons is a more efficient pathway to the CNS than via the blood circulation across the blood–brain barrier. Given that this neuronal translocation pathway was also demonstrated in nonhuman primates, it is likely to be operative in humans as well [52••,53].

In relation to the possibility that UFPs translocate from the lungs into the blood circulation, various studies have been conducted in different animal models. The amount of UFPs that translocated into blood and extrapulmonary organs differed amongst these studies [54–57]. Recent studies [58••,59,60] have provided morphological data illustrating that inhaled particles are transported into the pulmonary capillary space,

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presumably by transcytosis. It has also been shown that, following intranasal delivery, polystyrene microparticles (1.1 μm) can translocate to tissues in the systemic compartment [61].

In an ex-vivo model of isolated perfused rat lungs, lacking both lymph flow and recruited inflammatory cells [62•], it was confirmed that UFPs can translocate from the lung into the circulation, when pulmonary microvascular permeability was increased by pharmacological mediation (H_2O_2, vascular histamine administration). Using a similar model in rabbit lungs, we found a passage of UFPs from the blood vessels to the alveolar spaces, but only after histamine administration [63•].

The issue of particle translocation in humans is still controversial. In a recent study using an aerosol of technetium-99m-labelled carbon particles (10 nm in diameter, but these rapidly formed aggregates of 100 nm in diameter in the inhaled aerosol), no translocation into the systemic circulation could be demonstrated, and no significant radioactivity was found over the liver. The nature of the radioactivity found in blood (4.4%) consisted mainly of pertechnetate, as analysed by thin layer chromatography [64•]. These results contrast with previous findings also based on inhaling an aerosol of technetium-99m-labelled carbon particles [65], in which particle-bound radioactivity (also assessed by thin-layer chromatography) was detected in blood after 1 min, reaching a maximum between 10 and 20 min, and remaining at this level up to 60 min. Gamma camera images showed substantial radioactivity over the liver and other areas of the body. The discrepancies between these two studies may be related to the chromatography technique or the size of the inhaled particles. Further studies with other types of radioactive labelling should clarify the issue.

Effect of particles on thrombogenesis

The effects of particles on thrombosis constitute a relevant cardiovascular endpoint. Using a hamster model of vascular thrombosis in the femoral vein, we found a marked increase in prothrombotic tendency after intravenous injection of positively charged (amine-modified) polystyrene particles of 60 nm diameter, which resulted, at least in part, from platelet activation [66]. Similar effects were obtained after the intratracheal administration of these positively charged polystyrene particles [67]. In complete agreement with our findings, Silva *et al.* [68•], who used a rat model of ear vein thrombosis, also observed a significant dose-dependent enhancement of thrombus formation after intravenous and intratracheal administration of positively charged polystyrene particles but not with negatively charged polystyrene particles of the same size.

Using real pollutant particles, that is DEPs, we showed a marked pulmonary inflammation, enhanced venous and arterial thrombosis and platelet activation within 1 h of their deposition in the lungs [69]. The prothrombotic tendency, activation of circulating blood platelets, as well as lung inflammation persisted up to 24 h after instillation [70]. Pretreatment with an H1-receptor antagonist prevented the effects at 6 and 24 h, but the prothrombotic tendency observed 1 h after DEP exposure did not appear to correlate with pulmonary inflammation. The latter is compatible with direct platelet activation by DEPs (or their constituents), having penetrated into the circulation [70]. In subsequent experiments, we found that 24 h after DEP exposure, pretreatment with dexamethasone or with cromoglycate blocked the DEP-induced pulmonary inflammation, prothrombotic events and histamine release in bronchoalveolar lavage fluid and plasma. We concluded that the systemic inflammatory and prothrombotic effects found 24 h after DEP administration are secondary to lung inflammation, and that they can be prevented by mast cell stabilization [71]. More recently, using silica particles, we provided novel evidence for a critical role of macrophage–neutrophil cross-talk during lung inflammation induced by particles, leading to the release of neutrophil elastase into the systemic circulation. We found that neutrophil enzymes may be responsible for the priming of platelet activation and contribute to the development of a thrombotic tendency, when such primed platelets encounter a (mildly) injured vessel wall [72••]. Although the mechanism involved in the effect of silica particles is different from that triggered by DEPs, this study provided further evidence that inflammation is an integral component of thrombosis.

In support of this concept, it has been shown, using repeated exposures to particulate air pollution, that PM_{10} increased the volume and advanced the phenotype of the coronary atherosclerotic lesions in Watanabe heritable hyperlipidaemic rabbits [73]. Moreover, Sun *et al.* [74••] showed, in an $apoE^{-/-}$ mouse model, that long-term exposure to low concentrations of $PM_{2.5}$ altered vasomotor tone, induced vascular inflammation, and potentiated atherosclerosis.

Some in-vitro studies have evaluated the possible mechanisms of translocation and the vascular effects of particles. Geys *et al.* [75•] showed that nanoparticles are capable of translocating from the apical to the basolateral side of a confluent culture of Calu-3 cells. Studies with human vein endothelial cells have shown that urban particles are able to induce the expression of adhesion molecules such as E-selectin, ICAM-1, vascular cell adhesion molecule 1 and platelet–endothelial adhesion molecule 1 [76,77]. These effects appear to be partially related to the presence of endotoxin in the insoluble

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fraction [76], while the PM_{10} particles seem to induce a stronger effect than the $PM_{2.5}$ fraction [77]. Urban particles were also shown to induce oxidative stress (H_2O_2 production, activation of extracellular signal regulated kinase 1/2 and p38 mitogen-activated protein kinase) in human pulmonary endothelial cells [78••].

Conclusion

The adverse effects of exposure to particulate matter are manifested from early life onwards and at different levels. So far, the effects on lung function, COPD, allergic illness and lung cancer have been reasonably reproduced at an experimental level with regard to systemic, cellular and molecular effects. The importance of particulate matter translocation, both *in vivo* and *in vitro* has become a hot topic. The interactions between different cells have shown that the previous approach studying single cell lines may underestimate the effect of particulate matter. Regardless of the great effort to explain the impact of particulate matter on public health and at the experimental level, we are facing a great challenge to control the emission and composition of particulate matter. This is of special interest in mega cities, like Mexico City, presenting an average level of PM_{10} of 50 $\mu g/m^3$ influenced by industry, traffic and natural sources.

Acknowledgements

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 143).

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