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Interaction of matrix metalloproteinases with pulmonary pollutants

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ABSTRACT: An air pollutant consists of any atmospheric substance that may harm humans, animals, vegetation or material. Various air pollutants have been reported, differing in their physicochemical characteristics. They can be grouped into four categories: gaseous pollutants (*e.g.* ozone, sulfur dioxide, oxides of nitrogen, carbon monoxide and volatile organic compounds), persistent organic pollutants, heavy metals (*e.g.* cadmium, lead and mercury) and particulate matter (coarse, fine and ultrafine). These pollutants can reach the respiratory system, eliciting pulmonary and/or systemic effects. These effects include inflammation, tissue remodelling and carcinogenesis: all phenomena where matrix metalloproteases (MMPs) play critical roles, given their broad effects on matrix remodelling and modulation of inflammation and cell signalling. Moreover, since expression and activity of MMPs can be induced by such stimuli, the hypothesis has been raised that MMPs could be involved in the health effects of pollutants. Until now, the implication of MMPs in these effects has been studied only for some pollutants and for a restricted selection of MMPs (mainly MMP-1, -2, -9 and -12), while evidence for a link between MMP induction/activation and health effects remains scarce. A larger number of studies is, therefore, needed in order to better understand the implication of MMPs in health effects associated with air pollution.

KEYWORDS: Diesel, lung and systemic disease, matrix metalloproteinases, particulate exposure

An air pollutant consists of any atmospheric substance that may harm humans, animals, vegetation or material. As far as humans are concerned, an air pollutant may pose a present or potential hazard to human health or may cause or contribute to an increase in mortality or serious illness. The determination of whether or not a substance poses a health risk to humans is based on clinical, epidemiological and/or animal studies, which demonstrate that exposure to a substance is associated with health effects [1]. Since the respiratory system is in contact with the atmosphere, respiratory consequences of direct exposure to different atmospheric compounds are of great concern. Moreover, indirect systemic effects secondary to respiratory exposure to pollutants are increasingly recognised.

Progressive changes in atmospheric composition and the appearance of atmospheric pollutants are primarily due to the combustion of fossil fuels, used for the generation of energy and transportation. Various air pollutants have been reported, differing in their physicochemical characteristics. They can be grouped into four categories: 1) gaseous pollutants (*e.g.* ozone, sulfur dioxide (SO_2), oxides of nitrogen (NO_x), carbon monoxide (CO) and volatile organic compounds (VOCs)); 2) persistent organic pollutants (POPs); 3) heavy

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metals (*e.g.* cadmium, lead and mercury); and 4) particulate matter (coarse, fine and ultrafine).

Gases and aerosols of organic, metal and particulate pollutants can reach the respiratory system, eliciting pulmonary and/or systemic effects. Among these effects are inflammation and carcinogenesis, both phenomena where matrix metalloproteinases (MMPs) play critical roles, given their ability to modulate inflammatory mediators' effects and broad effects on matrix remodelling [2].

Since air pollutants represent a heterogeneous group of compounds with different effects on the respiratory system, it is difficult to present their effects on MMPs in an integrated way. Therefore, in this review, we will describe first the main respiratory and systemic effects of pollutants after respiratory exposure, focusing on the effects that could result from MMP involvement. We will then analyse separately the specific effects of each class of pollutants on the different MMPs. Finally, we will discuss some common aspects of the data analysed, especially in connection with the mechanisms and the consequences of MMP induction or activation after exposure to the different pollutants.

MAIN PATHOLOGICAL EFFECTS OF PULMONARY POLLUTANTS

For decades, outdoor air pollution has been known to cause clinically significant adverse health effects [3]. As an example, in London, UK, in December 1952, a 3-day fog episode resulted in 4,000 excess deaths during the two following weeks, as well as increased morbidity for respiratory and cardiovascular reasons [4].

Respiratory effects

Numerous studies describe that all types of air pollution, at high concentration, can functionally affect the respiratory system, even after a short-term exposure [3]. In addition, effects are also observed with long-term exposure to lower pollutant concentrations. These effects include increased mortality and morbidity in the general population, diminution of lung function in adults, as well as diminution of lung function in children raised in highly polluted areas [5–9]. As an example, chronic exposure to ozone and certain heavy metals reduces lung function [10]. The respiratory effects of pollutants also include inflammation, which can lead to tissue remodelling in the case of chronic exposure, *e.g.* emphysema-like lesions have been observed in mice exposed to nitrogen dioxide (NO_2) [11] or cadmium [12]. Additionally, in patients with previously existing lung lesions or lung diseases, pollutant-initiated inflammation will worsen their condition [13]. The effects of pollutants are related to the nature of the pollutant and its reactivity against biological targets. Moreover, the nature of the pollutant also dictates its site of deposition in the airways (*e.g.* large particles in proximal airways and small particles in distal airways and lung parenchyma [14]).

Respiratory exposure to pesticides, metals (*e.g.* hexavalent chromium), solvents (*e.g.* toluene), and air pollutants in general has been associated with an increased risk of lung cancer [15]. For example, in a European nested case–control study of nonsmokers and ex-smokers, residing near heavy traffic roads was linked to a 46% increase in lung cancer [16]. Another case–control study examining the risk of outdoor air pollution demonstrated that females living in the group of Taiwan municipalities with the highest levels of air pollution had a 28% increased risk of lung cancer [17].

Cardiovascular effects

Emerging evidence from epidemiological studies suggests that air pollution may have a focused impact on cardiovascular health. In particular, exposure to traffic has been shown to be a stronger risk for acute myocardial infarction and proximity to roadways is more strongly associated with coronary artery calcification than are indices of particulate matter respiratory exposure [18, 19]. Although particulate matter has a definite toxic effect on the systemic vasculature in rodent models and in controlled human studies [20, 21], environmental exposure to particulate matter never occurs without concomitant exposure to numerous gaseous co-pollutants. Diseases of the systemic vasculature can manifest in many ways, and we have a growing appreciation that particulate matter air pollution may exacerbate atherosclerosis [22], hypertension [23] and diabetic vasculopathy [24].

Underlying mechanisms of pollutant effects

The spectrum of respiratory and vascular disorders induced by exposure to air pollutants is wide. However, common biological pathways, such as inflammation, oxidative stress and enzymatic remodelling of the extracellular matrix (for review see [25, 26]), are described as driving progression of disease. Since MMPs are key players in the phenomena, and since their expression and activity can be induced by such stimuli [27–29], it is highly probable that MMPs could be involved in these effects of pollutants.

MODULATION OF MMPS BY GASEOUS POLLUTANTS

Gaseous pollutants contribute to a great extent to composition variations of the atmosphere and are mainly due to combustion of fossil fuels [30]. They include ozone, SO_2, NO_2 and VOCs. The involvement of MMPs on the effects of gaseous pollutants was mainly described for ozone and SO_2.

Ozone

Ozone is a colourless, odourless, reactive gas composed of three oxygen atoms. It is found naturally in the earth's stratosphere, where it absorbs the ultraviolet component of incoming solar radiation that could be harmful to life on earth. Ozone is also found near the earth's surface, in the lower atmospheric layers, where pollutants emitted from human activities react in the presence of sunlight to form ozone. Principal pollutants involved in the reaction of ozone formation are NO_x, VOCs and CO.

Epidemiological studies have demonstrated a strong association between high ambient ozone concentration and respiratory and cardiovascular morbidity and mortality [31]. Ozone exposure elicits airway inflammation characterised by neutrophil accumulation and the liberation of multiple inflammatory mediators, cytokines and chemokines as an early inflammatory event [32, 33]. Ozone-induced activation of airway neutrophilic infiltration is likely to produce additional damage through the release of reactive oxygen species and endogenous proteolytic enzymes. Different studies have shown that ozone exposure in

mice induced and/or activated different MMPs. We will analyse separately the effects on different MMPs.

MMP-2

Only one study analysed the effects of ozone on MMP-2. In this study, a single exposure of Fisher-344 rats to ozone (0.4 or 0.8 ppm) for 4 h did not result in an increase in MMP-2 activity in bronchoalveolar lavage (BAL) fluid [34]. However, co-exposure to 0.8 ppm ozone and 50 $mg \cdot m^{-3}$ of particulate matter resulted in a significant increase in MMP-2 activity, whereas, as for ozone only, exposure to particulate matter alone did not induce any increase. Although THOMSON *et al*. [34] did not investigate the mechanism of this phenomenon, it is possible that the extent of oxidative stress produced from co-exposure to particles plus ozone, in excess of what is observed with ozone or particles alone [35], rapidly activated MMP-2 [36, 37].

With regard to the consequences of these phenomena, THOMSON *et al*. [34] stated that the increased activation of MMP-2 after co-exposure to ozone and particulate matter is in line with the enhanced septal remodelling [35] and thickening [38] that result from co-exposure to particulate matter and ozone, by comparison to the changes induced by the individual pollutants. Furthermore, they postulated that MMP-2 synthesised by alveolar macrophages could have effects other than extracellular matrix degradation, for example cleavage of big endothelin-1 produced by endothelial cells to produce a short endothelin-1 peptide.

MMP-9

KENYON *et al.* [39] showed that exposure of C57BL/6J mice to 1 ppm of ozone for three consecutive days for 8 $h \cdot day^{-1}$ resulted in an increase in MMP-9 activity, as well as an increase in neutrophils in the BAL fluid. The authors postulate a cause–effect relationship between the former and the latter phenomenon.

Similar results were reported by YOON *et al.* [40], who demonstrated that exposure of C57BL/6J mice to 0.3 ppm of ozone for 6, 24, 48 or 72 h resulted in increased MMP-9 mRNA expression in lung homogenates after 6 h, followed by an increase in lung protein expression at 24 h and activity in BAL fluid at 48 h. The increase at 48 h was paralleled by an increase in MMP-2 protein expression and activity.

Using MMP-9 deficient ($MMP\text{-}9^{-/-}$) mice, the authors demonstrated that deficiency in MMP-9 was associated with enhanced airway epithelial injury, neutrophil recruitment and permeability following ozone exposure. The increased neutrophil recruitment was correlated with increased levels of keratinocyte-derived chemokine and macrophage inflammatory protein (MIP)-2 protein, but not mRNA expression, in the $MMP\text{-}9^{-/-}$ mice relative to $MMP\text{-}9^{+/+}$ mice. These results are consistent with the hypothesis that enhanced ozone-induced injury in $MMP\text{-}9^{-/-}$ mice is related to a difference in post-transcriptional processing of these CXC chemokines in the airway. Indeed, several lines of molecular evidence have determined that proteolytic function of MMP-9 affects cytokine and chemokine levels as well as their activities. Supporting the results of YOON *et al.* [40], increased tissue neutrophil and inflammatory cell infiltration have been shown in $MMP\text{-}9^{-/-}$ mice in response to epithelial injury and chemokine administration [41, 42].

MMP-12

In contrast with the previous studies, in which animals were exposed to ozone for short periods, TRIANTAPHYLLOPOULOS *et al.* [43] showed that chronic, repeated exposures of BALB/c mice to 2.5 ppm of ozone (3-h exposures twice per week, over 3 and 6 weeks) induced a time-dependent increase in MMP-12 mRNA and protein expression. By contrast, a single 3-h exposure did not induce any change in MMP-12 expression.

In this chronic model of ozone exposure, TRIANTAPHYLLOPOULOS *et al.* [43] propose that the increased MMP-12 is a main mechanism of the emphysematous alterations observed in this model, as reported in cigarette-smoke-related experimental emphysema [44]. However, they did not investigate this issue.

In all of these studies, MMP induction or activation was observed in the context of an inflammatory response. Moreover, it was associated sometimes with lung injury and oedema [39] or tissue remodelling [25, 43], depending on the duration of ozone exposure. Finally, these studies also show that exposures to ozone shorter than 24 h are not able to induce protein expression or activate the different MMPs examined.

SO_2

Anthropogenic SO_2 is a pollutant present in automobile fumes. It results from the combustion of sulfur-containing fossil fuels (principally coal and heavy oils). The smelting of sulfur-containing ores, volcanoes and oceans represent its major natural sources. SO_2 may play a role in the exacerbation of airway disease symptoms.

Only one study has investigated the effect of SO_2 on MMPs, focusing on MMP-9. In this study, O'BRIEN *et al.* [45] examined the action of SO_2 on mucociliary transport in a frog palate epithelial injury model. They used sodium metabisulfite, which releases SO_2 on contact with water. Sodium metabisulfite dose-dependently increased MMP-9 activity in epithelial tissue and mucus. This was associated with a loss of ciliated cells in sodium metabisulfite-treated palates compared to controls with an intact ciliary blanket and with a reduced mucociliary clearance time. The authors proposed that MMP-9 played a major role in these phenomena, through an action on cell–cell or cell–matrix attachments resulting in the exfoliation of intact ciliated epithelial cells, which may contribute to a slowing of mucus clearance over the surface.

MODULATION OF MMPS BY POPS

POPs are organic chemicals that are persistent and widely distributed in the environment, have bioaccumulative properties and are toxic to humans and wildlife [46]. They include pesticides, as well as dioxins, furans and polychlorinated biphenyls. Generally, the generic term ''dioxins'' is used to cover different compounds formed during incomplete combustion and whenever materials containing chlorine (*e.g.* plastics) are burned. Emitted in the atmosphere, dioxins tend to deposit on soil and water but, being water insoluble, they do not contaminate ground water sources. Most dioxins in plants come from air and dust or pesticides and enter the food chain where they bioaccumulate due to their ability to be stably bound to lipids. The respiratory system can be exposed to some compounds belonging to the POPs category, which are

present in cigarette smoke or adsorbed on the surface of particulate matter.

In this context, WONG *et al.* [47] examined the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) *in vitro* in human airway epithelial cell lines and *in vivo* in mice in order to analyse whether activation of the arylhydrocarbon receptor (AhR) was involved. MMP expression was only analysed in mice. TCDD administration (15 $mg \cdot kg^{-1}$ intraperitoneal) induced a significant increase in MMP-2, -9 and -13 mRNA expression in whole lung homogenates 1–30 days after administration. These increases were not observed in mice lacking the AhR, showing a role of this receptor in MMP induction. Similar results were reported by ISHIDA *et al.* [48] in the T24 human urothelial carcinoma cell line. These are interesting observations, since they place the AhR as an important mediator of MMP induction by contaminants. Furthermore, the AhR is also involved in an inflammatory response elicited by TCDD, showing its critical role in the orchestration of cell responses to certain contaminants.

MODULATION OF MMPS BY HEAVY METALS

In this section, we will analyse the effects of soluble metals. Those of particulate, insoluble metals are described in the section concerning modulation of MMPs by particulate matter. The effect of two metals (nickel and cadmium) on MMPs has been reported in the literature.

During recent decades, a growing body of literature has suggested that nickel could contribute to tumour progression in human lung cancer [49]. A number of possible mechanisms, including the induction of oxidative stress, inhibition of DNA repair and epigenetic modification, have been described, through which even low-dose, short-term exposure to nickel might enhance uncontrolled cell growth and cancer development [50]. However, the direct effect of nickel on the invasive potential of human lung cancer cells and the underlying mechanism still remain unknown. In an *in vitro* study, XU *et al.* [51] evaluated modulation of MMP-2 and -9 expression by nickel on human lung cancer cell lines A549 and H1299 in the context of the analysis of their growing capacity and invasiveness. They demonstrated that nickel could significantly enhance the invasive potential of A549 and H1299 cells in a dose-dependent manner. This was accompanied by an elevated expression of interleukin (IL)-8, transforming growth factor-β and MMP-2 and MMP-9 proteins. They further demonstrated that modulation of the invasive potential involved the Toll-like receptor (TLR)4 and protein MyD88, but they didn't analyse the involvement of this pathway on MMP-2 and -9 induction. Interestingly, a recent study in a nonalcoholic steatohepatitis and liver fibrosis model in mice showed that liver MMP-2 induction was prevented in TLR4-deficient animals [52]. Therefore, this receptor could also play a role in MMP-2 induction in the context of nickel-induced tumoral invasive potential.

Cadmium fumes can induce acute and often fatal lung damage but also severe, widespread centrilobular emphysema [53]. Cadmium inhalation can occur in an occupational context for people working in battery manufacturing, metal soldering, plastic or other synthetic production, and welding, but can also be environment-related due to municipal waste, coal and mineral oil combustion, at the vicinity of metallurgy, petrochemical or paint industries. In order to characterise cadmium-induced lung inflammation and emphysema, FIEVEZ *et al.* [54] examined MMP-2 and -9/tissue inhibitor of metalloproteinase (TIMP)-1 and -2 imbalance in rats exposed to cadmium nebulisation. Such nebulisation induced a significant increase in BAL MMP-2 and -9 and TIMP-2 expression and/or activities, whereas TIMP-1 was not detectable in any BAL samples. These phenomena were concomitant with neutrophil and macrophage accumulation in BAL and emphysema development and were not modified by administration of the corticosteroid betamethasone. This study reinforces the link between MMP-2 and -9 and pulmonary emphysema, already demonstrated after cigarette smoke exposure.

MODULATION OF MMPS BY PARTICULATE MATTER

Particulate matter is the generic term used for the type of air pollutants consisting of complex and varying mixtures of particles suspended in the breathed air. These mixtures vary in size and composition, and are produced by a wide variety of natural and anthropogenic activities [55]. Major sources of particulate pollution are factories, power plants, refuse incinerators, motor vehicles, construction activity, fires and natural windblown dust. The size of the particles, defined by their aerodynamic diameter ($PM_{2.5}$ and PM_{10} are the terms given to particles with a 50% cut-off aerodynamic diameter of 2.5 μm and 10 μm, respectively), varies and different categories have been defined: coarse particles larger than 1 μm, fine particles smaller than 1 μm, and ultrafine particles smaller than 0.1 μm in aerodynamic diameter. These sizes determine their site of deposition in the respiratory tract: PM_{10} particles deposit mainly in the upper respiratory tract while fine and ultrafine particles are able to reach lung alveoli [14]. So far, no single component has been identified that could explain most of the particulate matter effects. Among the parameters that play an important role for eliciting health effects are the size and surface of particles, their number and their composition. There is strong evidence to support the hypothesis that ultrafine and fine particles are more hazardous than larger ones (coarse particles), in terms of mortality and cardiovascular and respiratory effects [1].

In addition, the metal content, the presence of polycyclic aromatic hydrocarbons (PAHs) and other organic components, such as endotoxins, mainly contribute to particulate matter toxicity. Here, we will analyse the effects of different types of particulate matter on MMPs.

Diesel exhaust particles

Diesel exhaust particles (DEPs) are composed of a carbonaceous core with adsorbed organic compounds, sulfates and trace elements. Soluble organic compounds, including PAHs, can represent $\leqslant 60\%$ of the mass of the particle. The production of DEPs by vehicular traffic is a major contributor to urban particulate matter air pollution [56].

Inhalation of DEPs is associated with cardiovascular diseases (*e.g.* atherosclerosis, arrhythmias and thrombosis) and respiratory diseases (*e.g.* chronic asthma, chronic obstructive pulmonary disease (COPD) and bronchial cancer), leading to an increase in mortality. Because of the large number of hazardous chemicals that are present on DEPs, their pathological

effects on airways and lungs are pleiotropic, as documented in numerous studies that have focused on various pathological mechanisms. Specifically, DEPs have been shown to increase the secretion of pro-inflammatory cytokines, to release phosphatidylcholine, to produce reactive oxygen species that lead to oxidative injury, and to induce DNA damage, any or all of which may compromise lung function (for review see [57]). Moreover, as aforementioned, some of these phenomena participate in MMP induction [27–29]. Different studies have examined whether DEPs modulate MMP activity and/or expression. As for ozone, we will present these studies according to the MMP analysed.

MMP-1

Three studies investigated MMP-1 modulation by DEPs. Chronologically, the first one, by DOORNAERT *et al.* [58], showed that DEPs downregulated protein expression of MMP-1 in the human bronchial epithelial line 16HBE14o- without any modification of MMP-2 and MMP-9 activity and TIMP-1 and -2 protein expression. These effects were observed at a DEP concentration of 100 $\mu g \cdot mL^{-1}$.

By contrast with this study, AMARA *et al.* [59] described induction of MMP-1 expression and activity in the human lung epithelial cell lines A549 and NCI-H292 after incubation with 10 $\mu g \cdot cm^{-2}$ (equivalent to 50 $\mu g \cdot mL^{-1}$) DEPs. These authors reported no modification of TIMP-1 and -2 expression.

Results similar to those reported by AMARA *et al.* [59] were published by LI *et al.* [60]. This group described in the BEAS-2B cell line (SV40-adenovirus-transfected immortalised human bronchial epithelial cells) and in primary human bronchial epithelial cells that incubation with 50 and 100 $\mu g \cdot mL^{-1}$ DEPs led to a dose-dependent increased transcriptional activation of the *MMP-1* gene and subsequent secretion of MMP-1. This mechanism was enhanced by the -1607GG polymorphism within the *MMP-1* promoter, which is present in at least one allele in ~75% of humans and forms a known ETS (E-twenty-six) transcription factor binding site [61]. Interestingly, no secretion of MMP-2, -3, -9, -10 and -13 and TIMP-1 and -2 was induced by DEPs.

The difference between the two studies showing MMP-1 induction and the one showing downregulation could be related to the examined cell types, the methodology for investigating MMP-1 activity (differences in ELISA techniques) and, most importantly, differences in DEP composition. Indeed, this is a complex issue because the concentration of organic components was clearly different in the two studies showing an increase in MMP-1. Furthermore, this concentration was lower in the DEPs used in the study showing downregulation of MMP-1 [58] compared to one of those showing upregulation [59]. Clearly, components other than organic compounds are probably involved in MMP-1 modulation by DEPs.

Concerning the mechanisms of MMP-1 induction, the two studies showing this effect of DEPs converge on the critical role of the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (ERK)1/2. However, whereas AMARA *et al.* [59] analysed the role of reactive oxygen species synthesised by the NADPH (reduced nicotinamide adenine dinucleotide phosphate) oxidase NOX4 on this phenomenon, the study by LI *et al.* [60] demonstrated for the first time the roles of *raf*, *ras* and β-arrestins. Interestingly, both signalling pathways could be complementary, since it has been shown that β-arrestins can modulate reactive oxygen species production by NOX proteins [62].

These two studies focused on the mechanisms of MMP-1 induction, and they did not investigate the consequences of such a phenomenon. However, both studies speculate on the relevance of their findings in terms of a predisposing effect of DEPs to lung diseases in which MMP-1 has proven to be involved: pulmonary emphysema and lung cancer [25].

MMP-9

Two studies investigated MMP-9 modulation by DEPs. ZHANG *et al.* [63] demonstrated that DEPs induced MMP-9 mRNA expression in the murine lung epithelial cell line C10. These authors focused the study on the effects of DEPs on signalling pathways, especially fra-1 (fos-related antigen), a heterodimeric partner of activator protein (AP)-1. They showed that DEPs induced *fra-1* expression, which in turn upregulated MMP-9 promoter activity in transient transfection assays. Furthermore, the authors showed enhanced fra-1 binding to a functional site of the MMP-9 promoter after DEP stimulation. Consistent with this, DEPs also upregulated MMP-9 promoter activity. These results extend previous data showing sustained activation of *fra-1* by various toxins, such as cigarette smoke, silica or asbestos, in lung cell types [64]. Furthermore, data from the literature suggest that fra-1 can modulate the expression of other MMPs after air pollutant exposure. Indeed, fra-1 has been shown to upregulate MMP-12 gene expression in the U937 human monocytic cell line [65], and to play a critical role in maintaining a high-level constitutive MMP-1 gene expression in melanoma cells [66].

Consistent with the study by ZHANG *et al.* [63], MATSUZAKI *et al.* [67] showed an increase in MMP-9 protein release from human neutrophils after incubation for 2 h with DEP extracts prepared on methanol. This phenomenon was concomitant with an increase in intracellular H_2O_2 levels and surface expression of CD11b, an adhesion molecule essential for neutrophil migration into tissues. The interesting information from this study is the effect of DEP extracts, which suggest a role of soluble components of DEPs, such as PAHs.

Metallic particles

Different particles of metallic nature are found as atmospheric pollutants and can induce adverse respiratory effects. It is well known that exposure to metal particles such as metal fumes can cause pulmonary alterations (inflammation and fibrosis) in exposed workers [68]. Furthermore, transition metals also play a key role in the health problems induced by particulate matter [69]. Few of the studies investigating these effects analysed involvement of MMPs. In a descriptive *in vivo* study, BEAVER *et al.* [70] showed an increase in the levels of pro-MMP-9 in BAL fluid, strongly correlating with the presence of neutrophils in the airways, in mice exposed chronically to particles of hexavalent chromium, a well-known pro-inflammatory and carcinogenic agent [71, 72]. No further studies on MMPs were performed in this work; the authors interpreted the increase in MMP-9 as reflecting the presence of activated neutrophils.

Particulate matter

In a very elegant study, COBOS-CORREA *et al.* [73] investigated the presence of active MMP-12 in macrophages from BAL from mice exposed to ambient PM10. Using a ratiometric fluorescence resonance energy transfer reporter specific for MMP-12 (LaRee1) that is lipidated and targeted to the plasma membrane, they showed that active MMP-12 was present in the membrane of macrophages of animals instilled with the PM10. Inactive MMP-12 was present in cell-free BAL fluid. This is the first demonstration of membrane-bound active MMP-12 in macrophages. The authors derive two main conclusions from these results: 1) the mechanism of the membrane location of active MMP-12 implies post-translational control of MMP-12 (for example, by other proteinases localised in the vicinity of the macrophage surface); and 2) the consequences of this location is that elastolytic damage by MMP-12 might be caused by direct contact of macrophages with the extracellular matrix. As the authors concluded, the level of stimulation of alveolar macrophages, together with their localisation and mobility, may constitute determining factors in the pathogenesis of inflammatory lung diseases in which MMP-12 is involved.

Subway particles

In many large cities, the subway system is an important source of atmospheric pollution; PM10 concentration in the atmosphere can be $\leqslant$1,000 $\mu g \cdot m^{-3}$ [74, 75], well above the recommended 50 $\mu g \cdot m^{-3}$ daily ambient air limit [76]. These particles are rich in iron. Evaluation of the potential health effects of such emissions is important because, in France for example, the Paris subway system hosts more than one million commuters daily [77]. However, the effects of subway particles in the respiratory system are poorly known. In order to examine this issue, BACHOUAL *et al.* [78] exposed murine macrophages (RAW 264.7 cell line) and C57BL/6 mice to $\leqslant$10 $\mu g \cdot cm^{-2}$ and 100 μg, respectively, of subway particulate matter or ''reference'' materials (titanium dioxide (TiO_2), carbon black [79] and DEPs) over 24 h. They showed that noncytotoxic concentrations of subway particulate matter, but not of the other particles, induced a three-fold increased expression of MMP-12 mRNA both *in vitro* and *in vivo* (a transient increase in the latter). This was accompanied by a parallel increase in markers of oxidative stress (haem oxygenase-1 expression) and inflammation (tumour necrosis factor-α and MIP-2 production). *In vitro* experiments showed that particulate matter effects partially involved particulate matter iron. This is original information, little developed in other studies involving particles.

Manufactured nanoparticles

Nanotechnology includes the design, characterisation, production and application of structures, devices and systems by controlling shape and size at the nanometre scale [80]. These technologies directly improve our lives in areas as diverse as engineering, information technology and diagnostics. Nanomaterials are the building blocks of this new technology and comprise a range of different morphologies, including nanotubes, nanowires, nanofibres, nanodots and a range of spherical or aggregated dendritic forms. A nanoparticle is defined as an object having three dimensions $<$100 nm. Although both possess similar dimensions, nanoparticles fabricated for their particular properties in the context of nanotechnologies (called manufactured nanoparticles (MNPs)) are usually differentiated from ultrafine particles found in the atmosphere, which have different sources as detailed previously. Moreover, ultrafine particles are complex, usually containing different molecules adsorbed at their surface.

Some of the properties of nanomaterials that are unique and beneficial for technological applications may also endanger human health, inducing cyto- and genotoxic effects, inflammation and even cancer [80]. Inflammatory effects are particularly important. Free radical activity or oxidative capacity of MNPs might be essential for provoking these inflammatory responses. Recently, different investigators analysed the effects of MNPs on MMPs in the context of analysing the mechanisms of their inflammatory effects.

The physicochemical features of nanomaterials that account for their deleterious health effects include a large ratio of surface area to mass and associated increased surface reactivity, altered physicochemical properties such as changes in melting point or solubility, and electrical conductivity or changes (*e.g.* in the crystalline structure of the materials). Therefore, a detailed evaluation of these characteristics is critical in order to understand the mechanisms by which nanomaterials elicit biological responses. We will, therefore, analyse the effects of MNPs on MMPs according to their chemical nature.

With the development of nanotechnology, a large number of transition metal nanoparticles have been or will be developed and produced as new formulations with surface properties to meet novel demands. Since, as stated previously, exposure to metal particles can cause pulmonary alterations (inflammation and fibrosis) the analysis of pulmonary toxicity associated with metallic MNPs is an important issue. Although it was not directly related to the lung, a study by WAN *et al.* [81] provides interesting information about MMP regulation by metallic MNPs in monocytes. This information could be relevant to pulmonary pathophysiology, since one can expect similar results in pulmonary macrophages, which are key cells in the lung response to foreign particles. WAN *et al.* [81] compared the ability of a noncytotoxic concentration of cobalt (Co) and TiO_2 MNPs (5 $\mu g \cdot mL^{-1}$) to induce the activity and transcription of MMP-2 and -9 in the human monocyte cell line U937. They expected a higher effect of Nano-Co compared with Nano-TiO_2, since they showed in a previous study that Nano-Co caused greater lung injury and inflammation than Nano-TiO_2, although they have similar diameters [82]. The results of the study confirmed the authors' hypothesis, since Nano-Co, but not Nano-TiO_2, induced MMP-2 and -9 activity and mRNA expression. Furthermore, Nano-Co decreased mRNA expression of TIMP-2. These modifications involved oxidant signalling mechanisms, since 1) Nano-Co, but not Nano-TiO_2, induced cellular oxidative stress, and 2) the effects of Nano-Co were prevented by antioxidants. Moreover, using pharmacological tools, the authors demonstrated that the effects of Nano-Co involved the AP-1 and tyrosine kinase pathways. However, they did not demonstrate that oxidants mediated these last phenomena. In accordance with these results, MORIMOTO *et al.* [83] showed no induction of MMP-2 and TIMP-2 mRNA 1 month after rat exposure to aerosolised TiO_2 MNPs. Therefore, it appears that TiO_2 MNPs do not induce

MMP-2, and that induction by Co MNPs involves oxidant-mediated signalling.

Amorphous silica is another harmful nanoparticle. Individuals may be exposed to substantial quantities of synthetic amorphous silica at the workplace, as these materials are widely used in many industries and for various applications, *e.g.* fillers in the rubber industry, in tyre compounds, and as free-flow and anti-caking agents in powder materials. In addition, synthetic amorphous silicas are found in toothpaste additives, paints, silicon rubber, insulation material, liquid systems in coatings, car undercoats and cosmetics [84]. However, despite their widespread use in industry, their toxicities and toxic mechanisms are not well understood. CHOI *et al.* [85] investigated the degree of pulmonary fibrosis and the expression of fibrogenic mediators, including MMPs, in mice exposed intratracheally to a suspension of ultrafine amorphous silica and sacrificed 24 h, 1, 4 and 14 weeks later. They found that the mRNA and protein expression of MMP-2, -9 and -10 and TIMP-1 in lung tissues was significantly elevated at 24 h and 1 week post-instillation, although these levels decreased to near the control range at 4 and 14 weeks except for MMP-2. These changes were associated with a parallel transient alveolar epithelial thickening and pulmonary fibrosis and induction of the expression of inflammatory cytokines IL-4, IL-10, IL-13 and interferon-γ. This descriptive study demonstrates induction of several MMPs by amorphous silica in the context of an inflammatory response.

INDUCTION OF MMPS BY COMPLEX MIXTURES (GASES AND PARTICLES)

In parallel with the studies on selected air contaminants, other investigations have evaluated the respiratory effects of mixtures of different pollutants, which more closely represent real life exposure than exposure to individual pollutants.

In order to investigate the mechanisms of cardiovascular disease exacerbations induced by traffic-related pollutants, LUND *et al.* [86] examined modulation of vascular MMP-2 and -9 expression and activity by gasoline engine exhaust in a well-characterised model of vascular toxicity, the apolipoprotein E knockout ($ApoE^{-/-}$) mouse. Animals were exposed to gasoline engine exhaust (60 $\mu g \cdot m^{-3}$ particulate matter whole exhaust) or filtered air for 6 $h \cdot day^{-1}$ for a period of 1 or 7 days. The main results of the study show activation of vascular MMP-2 and -9 within 24 h of exposure to gasoline engine exhaust, followed by gene induction of the two MMPs over the following week, leading to *de novo* synthesis of additional proteins and prolonged maintenance of the vascular MMP response. These last findings were associated with vascular oxidative stress and endothelin-1 mRNA increase. MMP-2 gene induction was secondary to vascular oxidative stress, whereas MMP-9 induction was a consequence of oxidative stress and also of activation of the endothelin receptor A. Furthermore, vascular MMP-9 induction was associated with elevated levels of MMP-9 protein in the plasma of exposed mice. Interestingly, no markers of oxidative stress were detected in the lungs of exposed animals, thereby indicating that the observed vascular effects of gasoline engine exhaust may be independent of pulmonary oxidative stress pathways. The clinical relevance of these animal data was analysed in humans exposed for 2 h to diesel engine exhaust. These subjects displayed a significant increase in plasmatic levels of MMP-9, endothelin-1 and NO_x after exposure to diesel exhaust compared to air, thus confirming the translational relevance of the mouse model.

In order to better understand the role of the different components of gasoline exhaust on MMP induction and activation, the same group of investigators analysed the comparative effects of various gases representative of gasoline exhaust on vascular toxicity and MMP-9 expression and activity [87]. Exposure atmospheres included gasoline and diesel engine exhaust, hardwood smoke, a simulated ''down-wind'' coal combustion atmosphere (SDCCA), biogenically derived secondary organic aerosols (SOAs), and individual combustion source gases (nitric oxide (NO), NO_2 and CO). The authors used the $ApoE^{-/-}$ mouse to assess comparative responses to these atmospheres. The animals were exposed for 6 $h \cdot day^{-1}$ for 7 days. 18 h after the end of exposure, the aorta and blood were sampled for analysis.

In this study by CAMPEN *et al.* [87], the diesel exhaust, SDCCA, hardwood smoke and SOA exposures were conducted at matching particulate matter concentrations (300 $\mu g \cdot m^{-3}$). Because gasoline exhaust contains a very low mass of particulate matter, mice were also exposed to the highest concentration used in a previous study (60 $\mu g \cdot m^{-3}$ particulate matter).

The main results of the study by CAMPEN *et al.* [87] indicate that gasoline and, to a lesser extent, diesel exposure induced MMP-9 mRNA expression and activity in the aorta. Interestingly, whereas gasoline exposure also induced an increased gelatinase activity, such an effect was absent after exposure to diesel combustion. By contrast, hardwood smoke increased gelatinase activity without any increase in MMP-9 mRNA expression. The other compounds did not change MMP-9 expression or activity.

MMP-9 induction by gasoline and diesel paralleled the capacity of these atmospheres to induce lipid peroxidation, suggesting a causative role of oxidative stress on MMP-9 induction. However, both CO and NO gases, at concentrations close to those found in gasoline, induced MMP-9 expression without oxidative stress, pointing towards other mechanisms of MMP-9 induction. Moreover, both CO and NO activated MMP-9 as gasoline did, stressing the probable involvement of these gases on the effects of gasoline on MMP-9.

The authors of these two studies analysed the consequences of MMP-9 activation in terms of its potential role in destabilising vulnerable atherosclerotic plaque progression [88], which may be a predisposing factor for acute myocardial infarction. Furthermore, elevated plasma MMP-9 has been identified as a predictor of cardiovascular mortality [89].

Regarding the effects of combined gases and particles on MMPs, another group of investigators also examined whether MMPs are involved in woodsmoke-induced pulmonary emphysema [90]. Indeed, exposure to domestic woodsmoke and other biomass solid fuels used as domestic heating and cooking fuels is an important cause of COPD, especially in developing countries. RAMOS *et al.* [90] developed a sequential model of subchronic exposure to woodsmoke in guinea pigs (60 $g \cdot day^{-1}$ of pine wood, 5 $days \cdot week^{-1}$ for 1–7 months) and

analysed histological features, elastolysis, collagenolysis, gelatinolysis and expression of MMP-1, -2 and -9. Histological analysis after 4–7 months in smoke-exposed guinea pigs showed alveolar mononuclear phagocyte and lymphocytic peribronchiolar inflammation and mild-to-moderate emphysematous lesions. A higher elastolytic and collagenolytic activity in BAL macrophages and lung tissue homogenates was observed. The authors postulated that the elastolytic activity was probably due to MMP-12, since it was inhibited by EDTA. Lung MMP-2 and -9 activity and mRNA expression were increased at 4 and 7 months. MMP-9 protein was localised in epithelial cells and macrophages in woodsmoke-exposed animals, along with expression of MMP-1. Collectively, these results are similar to those induced by cigarette smoke exposure in guinea pigs [91] and suggest a role of MMP-1, -2, -9 and probably -12 in woodsmoke-induced pulmonary emphysema.

DISCUSSION

Mechanisms and consequences of MMP modulation by air pollutants

Most of the studies focused on MMPs and air pollutants have examined their effects on MMP-2 and -9, some of them on MMP-1 and only a few on MMP-12; other MMPs were not or were only extremely rarely studied (see table 1 for an overview). The studies analysed constitute a heterogeneous group of work when regarded in relation to the mechanisms of MMP induction and their implications in the effects of air pollutants. Only a small number of publications were specifically dedicated to investigation of the molecular mechanisms of induction/activation of MMPs or the physiological consequences of these phenomena. Indeed, in most of the studies, MMPs were investigated more as players of the pathophysiological process examined (pulmonary inflammation or remodelling, or carcinogenesis) than as specific study objects.

A summary of the main mechanistic data about MMP induction by pollutants is as follows. 1) MMP-2 and -9 can be induced *via* TLR4 (nickel exposure) [92] or secondary to oxidative signalling (gasoline exposure) [86]. In addition, MMP-9 can be induced *via* fra-1, a heterodimeric partner of AP-1, which binds to and activates the MMP-9 promoter (DEP exposure). 2) MMP-1 mRNA and protein expression can be induced *via* the MAPK ERK1/2, secondary to the action of NOX4 or β-arrestin (DEP exposure). 3) Active MMP-12 is located in macrophage membranes after exposure to PM_{10} [73].

Can these data be extrapolated to other systems or to other MMPs? The answer is positive in some cases. Concerning MMP-2 and -9 induction *via* TLR4 after nickel and gasoline exposure, other studies have reported similar findings in different cell types, such as astrocytes [93], human aortic smooth muscle cells [94] and synovial membrane cultures after different kinds of stimuli [95]. Moreover, a recent study in a nonalcoholic steatohepatitis and liver fibrosis model in mice showed that liver MMP-2 induction was prevented in TLR4-deficient animals [52]. Induction of MMP-1 has also been reported to be secondary to TLR4 activation after cigarette smoke exposure, *via* a MyD88/IRAK1 (IL-1 receptor-associated kinase 1) pathway [96]. Therefore, the involvement of TLR4 in MMP-2 and -9 induction by some air pollutants appears as a mechanism shared by other stimuli.

Similar considerations can be applied to the involvement of *fra-1* on MMP-9 induction by DEPs, as fra-1 has been shown to upregulate MMP-12 gene expression in the U937 human monocytic cell line [65], and to play a critical role in maintaining a high-level constitutive MMP-1 gene expression in melanoma cells [66].

A role of NOX proteins in modulating other MMPs than MMP-1 has also been reported, but less abundantly than the role of TLR4. NOX2 has been reported to mediate MMP-9 mRNA induction *via* reactive oxygen species production and ERK1/2 activation in macrophages [97]. NOX proteins have been also shown to control MMP-13 mRNA induction in human articular chondrocytes, but the nature of the involved NOX was not investigated in this study.

Collectively, this analysis shows that data generated in the field of MMP modulation by air pollutants provide some interesting information for better understanding MMP biology. In general, these data were also obtained in other models. Moreover, the amount of mechanistic information concerning MMP induction and/or activation is modest when compared to the amount of information available on MMPs and air pollutants.

Similarly to the mechanistic studies on MMP induction and/or activation, very few publications have examined the biological/physiological consequences of MMPs on pulmonary alterations induced by pollutants. Strictly speaking, only one study examined the consequences of MMP-9 induction on airway epithelial injury, neutrophil recruitment and permeability following ozone exposure by using MMP-$9^{-/-}$ mice. As described earlier in this review, this study found an enhanced ozone-induced injury in MMP-$9^{-/-}$ mice, which was related to a difference in post-transcriptional processing of CXC chemokines in the airway. Indeed, several lines of molecular evidence have determined that proteolytic function of MMP-9 affects cytokine and chemokine levels as well as their activities. Supporting these data, increased tissue neutrophil and inflammatory cell infiltration have been shown in MMP-$9^{-/-}$ mice in response to epithelial injury and chemokine administration [41, 42].

Clinical relevance of MMP modulation by air pollutants

Almost no translational or clinical studies focused specifically on MMPs and air pollutants are available in the literature. One exception is the study by LI *et al.* [60], showing that a polymorphism of the MMP-1 promoter predisposes to an enhanced gene transcription after DEP exposure. Whether this phenomenon exists in the case of induction of other MMPs after exposure to air pollutants is not known. Similarly, no experimental or translational information is available concerning a potential role of MMPs in the reported aggravation of respiratory diseases after exposure to pollutants. Unpublished data from our laboratory (Inserm U955, Créteil, France) show that exposure to carbon black nanoparticles enhances MMP-12 mRNA and protein expression in alveolar macrophages in a murine model of elastase-induced emphysema. The clinical relevance of this finding deserves further investigation. A role of MMPs in aggravating pulmonary diseases especially

TABLE 1 Summary of studies examining matrix metalloproteinase (MMP) modulation by air pollutants

First author [ref.]	Air pollutant	Experimental model	MMP	Main results	Mechanisms of MMP induction
Thomson [34]	Ozone	Rats exposure	MMP-2	No change	
Kenyon [39]	Ozone	Mice exposure	MMP-9	Increased activity in BAL	
Yoon [40]	Ozone	Mice exposure	MMP-2 and MMP-9	Increased MMP-9 mRNA expression and MMP-9 and MMP-2 protein expression in lung and activity in BAL	Consequence: protective role of MMP-9 against ozone-induced injury
Triantaphyllopoulos [43]	Ozone	Mice exposure	MMP-12	Increased mRNA and protein expression in lung	
O'Brien [45]	Sulfur dioxide	Frog palate	MMP-9	Increased activity in epithelial tissue and mucus	
Wong [47]	Persistent organic pollutants	Human airway epithelial cell lines, mice exposure	MMP-2, MMP-9 and MMP-13	Increased mRNA expression in cells and in lung	Role of the arylhydrocarbon receptor
Xu [51]	Nickel	Human lung cancer cell lines	MMP-2 and MMP-9	Increased protein expression	
Fievez [54]	Cadmium	Rats exposure	MMP-2 and MMP-9	Increased activity in BAL	
Doornaert [58]	Diesel exhaust particles	Human bronchial epithelial cell line	MMP-1	Decreased protein expression	
Amara [59]	Diesel exhaust particles	Human lung cancer cell lines	MMP-1	Increased mRNA protein expression	Role of NADPH oxidase (NOX4) and ERK1/2
Li [60]	Diesel exhaust particles	Human bronchial primary cells and epithelial cell line	MMP-1	Increased mRNA protein expression	Role of β-arrestins, *raf*, *ras* and ERK1/2
Zhang [63]	Diesel exhaust particles	Murine lung epithelial cell line	MMP-9	Increased mRNA expression	Role of *fra-1*
Matsuzaki [67]	Diesel exhaust particles	Human neutrophils	MMP-9	Increased protein release	Similar effects by particles and organic extracts
Beaver [70]	Hexavalent chromium particles	Mice exposure	MMP-9	Increased pro MMP-9 levels in BAL	
Cobos-Correa [73]	Ambient particulate mater	Mice exposure	MMP-12	Increased active protein in BAL macrophages membrane	
Bachoual [78]	Subway particles	Murine macrophage cell line, mice exposure	MMP-12	Increased mRNA expression in cells and lung	Partial role of iron
Wan [81]	Cobalt and titanium dioxide nanoparticles	Human monocyte cell line	MMP-2 and MMP-9	Increased activity and mRNA expression with cobalt but not titanium nanoparticles	Role of oxidants in cobalt effects
Choi [85]	Amorphous silica nanoparticles	Mice exposure	MMP-2, MMP-9 and MMP-10	Increased expression in lung	
Lund [86]	Gasoline engine exhaust	Mice exposure (ApoE$^{+/+}$ and $^{-/-}$), human exposure	MMP-2 and MMP-9	MMP-2 and MMP-9 activation and protein induction in aorta, elevated MMP-9 protein levels in plasma in mice and humans	Role of oxidants
Campen [87]	Various gases representative of gasoline exhaust	Mice exposure (ApoE$^{+/+}$ and $^{-/-}$)	MMP-9	Increased activity and expression in aorta	
Ramos [90]	Woodsmoke	Mice exposure	MMP-1, MMP-2 and MMP-9	Increased activity and protein expression in lung (alveolar epithelial cells and macrophages)	

BAL: bronchoalveolar lavage; NADPH: reduced nicotinamide adenine dinucleotide phosphate; ERK: extracellular signal-regulated kinase; fra: fos-related antigen; Apo: apolipoprotein.

concerns conditions in which MMPs play critical roles. Since the MMPs mostly examined in the case of exposure to air pollutants (MMP-1, -2, -9 and -12) have been shown to be involved in different pulmonary diseases (fibrosis, COPD and lung cancer progression, among others [25, 26]), clinical consequences of their induction in terms of mechanisms of development or aggravation of these diseases are very likely. However, formal proof is still lacking.

PERSPECTIVES

Exposure to air pollutants has long been associated with deleterious health effects. However, the implication of MMPs in these effects has been studied only for some pollutants and a restricted number of MMPs, while evidence for the link between MMP induction/activation and health effects is still scarce. A larger number of studies is, therefore, needed in order to better understand the implication of MMPs in health effects associated with air pollution. Moreover, the appearance of new pollutants, such as MNPs, should trigger specific studies aimed at the evaluation of the role of MMPs in their potential health effects.

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STATEMENT OF INTEREST

None declared.

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