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► **To cite this version:**

Sophie Lanone, Maylis Dagouassat, Jorge Boczkowski. INTERACTION OF MATRIX METALLOPROTEINASES WITH PULMONARY POLLUTANTS. *European Respiratory Journal*, European Respiratory Society, 2012, 39 (4), pp.1021-1032. 10.1183/09031936.00195811 . inserm-03109423

HAL Id: inserm-03109423

<https://www.hal.inserm.fr/inserm-03109423>

Submitted on 13 Jan 2021

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**INTERACTION OF MATRIX METALLOPROTEINASES
WITH PULMONARY POLLUTANTS**

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Abstract

An air pollutant consists in 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, SO₂, NO_x, CO, Volatile Organic Compounds), persistent organic pollutants, heavy metals (e.g. cadmium, lead, mercury), and particulate matter (coarse, fine, ultrafine). These pollutants can reach the respiratory system, eliciting pulmonary and/or systemic effects. These effects include inflammation, tissue remodeling and carcinogenesis, all phenomena where matrix metalloproteases (MMPs) play critical roles given their broad effects of matrix remodeling and modulation of inflammation and cell signaling. Moreover, since expression and activity of MMPs can be induced by such stimuli, hypothesis has been raised that MMPs could be involved in health effects of pollutants. As for now, the implication of MMPs in these effects has been studied only for some pollutants, and a restricted amount of MMPs (mainly MMP-1, -2, -9, and -12), while the link between MMP induction/activation and health effects remains scarce. A larger amount of studies is therefore deeply needed to better understand the implication of MMPs in health effects associated to air pollution.

Key words : matrix metalloproteinases (MMP), lung and systemic disease, diesel, particulate exposure

Introduction

An air pollutant consists in 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 recognized.

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, SO₂, NO_x, CO, Volatile Organic Compounds).
2. Persistent organic pollutants (POPs).
3. Heavy metals (e.g. cadmium, lead, mercury).
4. Particulate Matter (coarse, fine, ultrafine).

Gases and aerosols of organic, metal and particulate pollutants can reach the respiratory system, eliciting pulmonary and/or systemic effects. Among them, inflammation and carcinogenesis, both phenomena where MMPs play critical roles given their ability to modulate inflammatory mediators' effects and broad effects on matrix remodeling [21].

Since air pollutants represent a heterogeneous group of compounds with different effects on the respiratory system, 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 MMPs involvement. We will then analyze separately the specific effects of each class of pollutants on the different MMPs. We will finally discuss some common aspects of the data analyzed, especially in connection with the mechanisms and the consequences of MMPs 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, England, in December 1952, a 3 days long-fog episode resulted in 4000 excess deaths during the 2 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]. Besides, effects are also observed with long-term exposure to lower pollutant concentrations. These effects include increase 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 remodeling in the case of chronic exposure; emphysema-like lesions have been observed in mice exposed to nitrogen dioxide [11] or cadmium [12]. Besides, in patients with already-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 particle 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 non-smokers 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 women 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 epidemiologic 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 (PM) respiratory exposure [18, 19]. Although PM has a definite toxic effect on the systemic vasculature in rodent models and in controlled human studies [20, 21], environmental exposure to PM 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 PM air pollution may exacerbate atherosclerosis [22], hypertension [23], and diabetic vasculopathy [24].

Underlying mechanisms of pollutants 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 remodeling of the extracellular matrix (see [25, 26] for review), 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 therefore highly probable that MMPs could thus be involved in these effects of pollutants.

Modulation of MMPs by gaseous pollutants

Gaseous pollutants contribute to a great extent in composition variations of the atmosphere and are mainly due to combustion of fossil fuels [30]. They include ozone, **SO₂**, NO₂ and volatile organic compounds (VOCs). The involvement of MMPs on the effects of gaseous pollutants was mainly described for ozone and **SO₂**.

a. Ozone

Ozone is a colorless, odorless 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 nitrogen oxides, VOCs, and carbon monoxide.

Epidemiologic studies have demonstrated a strong association between high ambient ozone concentration with respiratory and cardiovascular morbidity and mortality [31]. Ozone exposure elicits airway inflammation characterized by neutrophils 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 showed that ozone exposure in mice induced and/or activated different MMPs. We will analyze separately the effects on different MMPs.

MMP-2

Only one study analyzed the effects of ozone on MMP-2. In this study, a single exposure of Fisher-344 rats to ozone (0.4 or 0.8 part per million [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/m³ 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 the authors 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], activated rapidly MMP-2 [36, 37].

In regard to the consequences of these phenomena, the authors stated that the increased activation of MMP-2 after a co-exposure to ozone and particulate matter is in line with the enhanced septal remodeling [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 synthesized by alveolar macrophages could have effects other than extracellular matrix degradation, as for example cleavage of big endothelin-1 (ET-1) produced by endothelial cells to produce a short endothelin-1 peptide.

MMP-9

Kenyon and coworkers [39] showed that C57BL/6j mice exposure to 1 ppm of ozone 3 consecutive days during 8h/day 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 and coworkers [40] who demonstrated that exposure of C57BL/6j mice to 0.3 ppm of ozone during 6, 24, 48 or 72 h resulted in an increased MMP-9 mRNA expression in lung homogenates after 6h, followed by an increase in lung protein expression at 24h and activity in BAL fluid at 48h. The increase at 48h was paralleled by an increase in MMP-2 protein expression and activity.

Using MMP-9 deficient (MMP-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 KC and MIP-2 protein, but not mRNA expression, in the MMP-9 $-/-$ mice relative to MMP-9 $+/+$ mice. These results are consistent with the hypothesis that enhanced ozone-induced injury in MMP-9 $-/-$ mice is related to a difference in posttranscriptional 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 and coworkers [40], increased tissue neutrophil and inflammatory cell infiltration have been shown in MMP 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 and coworkers [43] showed that chronic, repeated exposures of BALB/c mice to 2.5 ppm of ozone (3h exposures every 3 days per week, over 3 and 6 weeks) induced a time-dependent increase in MMP-12 mRNA and protein expression. By contrast, a single 3h exposure did not induce any change in MMP-12 expression.

In this chronic model of ozone exposure the authors 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, MMPs induction or activation was observed in the context of an inflammatory response. Moreover, it was associated some times with lung injury and edema [39] or tissue remodeling [25, 43], depending on the duration of ozone exposure. Finally, these studies also show that exposures to ozone shorter than 24h are not able to

induce protein expression or activate the different MMPs examined.

b. SO₂

Anthropogenic SO₂ is a pollutant present in automobile fumes. It results from the combustion of sulphur-containing fossil fuels (principally coal and heavy oils). The smelting of sulfur-containing ores, volcanoes and oceans represent its major natural sources. SO₂ may play a role in the exacerbation of airway disease symptoms.

Only one study so far investigated the effect of SO₂ on MMPs, focusing on MMP-9. In this study, O'Brien and collaborators [45] examined the action of SO₂ on mucociliary transport in a frog palate epithelial injury model. They used sodium metabisulphite (MB), which releases SO₂ on contact with water. MB dose-dependently increased MMP-9 activity in epithelial tissue and mucus. This was associated with a loss of ciliated cells in MB palates compared to controls with an intact ciliary blanket and with a reduced mucociliary clearance time. The authors propose that MMP-9 played a major role on 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 persistent organic pollutants (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 PCBs. 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 bio-accumulate due to their ability to be stably bound to lipids. The respiratory system can be exposed to some compounds belonging to the POPs, which are present in cigarette smoke or adsorbed on the surface of PM.

In this context, Wong and coworkers [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 analyze if its effects involve or not activation of the

arylhydrocarbon receptor (AhR). MMPs expression was only analyzed in mice. TCDD administration (15 mg/kg, intraperitoneal) induced a significant increase in MMP-2, -9 and -13 mRNA expression in whole lung homogenates from 1 to 30 days after administration. These increases were not observed in mice lacking the AhR showing a role of this receptor in MMPs induction. Similar results were reported by Ishida and coworkers [48] in the T24 human urothelial carcinoma cell line. These are interesting observation since they place the AhR as an important mediator of MMPs induction by contaminants. Furthermore, the AhR is also involved in an inflammatory responses elicited by TCDD, showing thus its critical role in the orchestration of cell responses to certain contaminants.

Modulation of MMPs by heavy metals

In this section we will analyze 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 was reported in the literature.

During recent decades, a growing body of literature suggested that nickel could contribute to tumor 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 and coworkers [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 IL-8, TGF- β , and MMP-2 and MMP-9 protein expression. They further demonstrated that modulation of the invasive potential involved the toll like receptor 4 (TLR4) and protein MyD88, but they didn't analyze 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 fume 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, 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 characterize cadmium-induced lung inflammation and emphysema, Fievez and collaborators [54] examined MMP-2, -9/TIMP-1,-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 neutrophils and macrophages 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 (PM) is the generic term used for the type of air pollutants consisting of complex and varying mixtures of particles suspended in the breathing 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 varies (PM_{2.5} and PM₁₀ for aerodynamic diameter smaller than 2.5 μm and 10 μm respectively) and different categories have been defined: coarse particles, larger than 1 μm , fine particles, smaller than 1 μm , and ultrafine particles (UFP), smaller than 0.1 μm in aerodynamic diameter. These sizes determine their site of deposition in the respiratory tract: PM₁₀ particles deposit mainly in the upper respiratory tract while fine and ultra fine particles are able to reach lung alveoli [14]. So far, no single component has been identified that could explain most of the PM 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 that UFP 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 PAHs and other organic components such as endotoxins, mainly contribute to PM toxicity.

We will analyze the effects of different types of PM on MMPs.

a. 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 up to 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, thrombosis) and respiratory diseases (e.g., chronic asthma, COPD, bronchial cancer), leading to an increase in mortality. Because of the large number of hazardous chemicals that are present on DEPs, their pathologic effects on airways and lungs are pleiotropic, as documented in numerous studies that have focused on various pathologic mechanisms. Specifically, DEPs have been shown to increase the secretion of pro-inflammatory cytokines, the release phosphatidylcholine, to produce ROS that lead to oxidative injury, and to induce DNA damage, any or all of which may compromise lung function (see [57] for review). Moreover, as stated earlier, some of these phenomena participate in MMPs induction [27-29]. Different studies examined if DEPs modulated MMPs activity and/or expression. As for ozone, we will present these studies according to the MMP analyzed.

MMP-1

Three studies investigated MMP-1 modulation by DEP. Chronologically, the first one, by Doornaert and colleagues [58] showed that DEPs (SRM 1650 from the National Institute of Standards and Technology, Gaithersburg, MD, USA) down regulated 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 concentration of 100 µg/ml of DEP.

By contrast with this study, Amara and associated [59] described induction of MMP-1 expression and activity in the human lung epithelial cell lines A549 and NCI-H292 after

incubation with 10 µg/cm² (equivalent to 50 µg/ml) DEPs particles (SRM 2975 from the National Institute of Standards and Technology, Gaithersburg, MD, USA). These authors reported no modification of TIMP-1 and TIMP-2 expression.

Results similar to those reported by Amara and coworkers [59] were published by Li and collaborators [60]. This group described in BEAS-2B cell line (SV-40 adenovirus-transfected immortalized human bronchial epithelial cells) and in primary human bronchial epithelial cells that incubation with 50 and 100 µg/ml DEP (provided by the Environmental Protection Agency, NC, USA) lead 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 approximately 75% of humans and forms a known ETS 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 2 studies showing MMP-1 induction and the one showing down-regulation could be related to the examined cell types, the methodology to investigate MMP-1 activity (differences in ELISA techniques) and, most importantly, differences in DEPs composition. Indeed, this is a complex issue because the concentration of organic components was clearly different in the 2 studies showing an increase in MMP-1. Furthermore, this concentration was lower in the DEPs used in the study showing down-regulation of MMP-1 [58] as compared to one of those showing up-regulation [59]. Clearly, components other than organic compounds are probably involved in MMP-1 modulation by DEPs.

Concerning the mechanisms of MMP-1 induction, the 2 studies showing this effect of DEPs converge on the critical role of the mitogen activated protein kinase (MAPK) ERK ^{1/2}. However, whereas Amara and coworkers [59] analyzed the role of ROS synthesized by the NOX4 NADPH oxidase on this phenomenon, the study by Li and coworkers [60] demonstrate for the first time the roles of *raf* and *ras* and β-arrestins. Interestingly, both signaling pathways could be complimentary since it has been shown that beta-arrestins can modulate ROS production by NADPH oxidase [62].

These 2 studies focused on the mechanisms of MMP-1 induction, and they did not investigate the consequences of such 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 and coworkers [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 signaling pathways, especially *fra-1*, 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 an enhanced *fra-1* binding to a functional site of the MMP-9 promoter activity after DEP stimulation. Consistently, DEPs also upregulated the 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 pollutants 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 above-cited study, Matsuzaki and collaborators [67] showed an increase in MMP-9 protein release from human neutrophils from 2 hours incubation with DEP extracts prepared on methanol. This phenomenon was concomitant with an increase in H₂O₂ intracellular levels and surface expression of CD11b, an adhesion molecule essential for neutrophil migration into tissues. The interesting information of this study is the effect of DEP extracts, which suggest a role of soluble components of DEP particles, such as aromatic polycyclic hydrocarbons.

b. 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 fume can cause pulmonary alterations (inflammation, fibrosis) in exposed workers [68]. Furthermore, transition metals also play a key role in the health problems induced by PM [69]. Few of the studies investigating these effects analyzed involvement of MMPs. In a descriptive *in vivo* study, Beaver and coworkers [70] showed an increase in the levels of pro-MMP-9 in BAL fluid, strongly correlating with the presence of neutro-

phils in the airways, in mice exposed chronically to particles of hexavalent chromium [Cr(VI)], 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.

c. Particulate Matter (PM)

In a very elegant study, Cobos-Correa and coworkers [73] investigated the presence of active MMP-12 in macrophages from BAL from mice exposed to ambient PM₁₀. Using a ratiometric FRET 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. An 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 drive two main conclusions of these results: 1) the mechanism of the membrane location of an active MMP-12 implies a post-translational control of MMP-12 (for example, by other proteinases localized in the vicinity of the macrophage surface), and 2) the consequences of this location is that elastolytic damage by MMP12 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 localization and mobility, may constitute determining factors in the pathogenesis of inflammatory lung diseases in which MMP-12 is involved.

d. Subway particles

In many large cities, the subway system is an important source of atmospheric pollution; PM₁₀ concentration in the atmosphere can be up to 1000 µg/m³ [74, 75] high above the 50 µg/m³ daily ambient air recommended limit [76]. These particles are reach in iron. The potential health effects of such emissions are important to evaluate because, in France for example, the Paris subway system hosts more than 1 million commuters daily (<http://www.ratp.fr>). However, the effects of subway particles in the respiratory system are poorly known. In order to examine this issue, Bachoual and coworkers [77] exposed murine macrophages (RAW 264.7 cell line) and C57Bl/6 mice up to 10 µg/cm² and 100 µg, respectively, of subway PM or “reference” materials (TiO₂, carbon black [78], and DEPs) over 24 hours. They showed that non-cytotoxic concentrations of subway PM, but not of the other particles, induced a 3-fold increased expression of MMP-12 mRNA both *in vitro* and *in vivo* (a transient increase in this last case). This was accompanied by a

parallel increase in markers of oxidative stress (HO-1 expression) and inflammation (TNF- α and MIP-2 production). *In vitro* experiments showed that PM effects partially involved PM iron. This is a original information, little developed in other studies involving particles.

e. Manufactured nanoparticles

Nanotechnology includes the design, characterization, production, and application of structures, devices and systems by controlling shape and size at the nanometer scale [79]. 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, nanofibers, nanodots and a range of spherical or aggregated dendritic forms. A nanoparticle is defined as an object having 3 dimensions < 100 nm. Although both posses similar dimensions, nanoparticles fabricated for their particular properties in the context of nanotechnologies (called manufactured nanoparticles, MNP) are usually differentiated from UFP found in the atmosphere, which has different sources as detailed previously. Moreover, UFP are complex, containing usually 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 [79]. Inflammatory effects are particularly important. Free radical activity or oxidative capacity of MNP might be essential for provoking these inflammatory responses. Recently different investigators analyzed the effects of MNP on MMPs in the context of analyzing the mechanisms of their inflammatory effects.

The physico-chemical 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 physic-chemical 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 to understand the mechanisms by which nanomaterials elicit biological responses. We will therefore analyze the effects of MNP 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, fibrosis) the analysis of pulmonary toxicity associated to metallic MNPs is an important issue. Although it was not directly related to the lung, a study of Wan and coworkers [80] provides interesting information about MMPs regulation by metallic MNPs in monocytes. This information can be relevant to pulmonary pathophysiology since one can expect close results in pulmonary macrophages, which are key cells in lung response to foreign particles. Wan and coworkers [80] compared the ability of a non-cytotoxic concentration of Cobalt (Co) and TiO₂ MNPs (5 µg/ml) 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 as compared to Nano-TiO₂ since they showed in a previous study that Nano-Co caused greater lung injury and inflammation than Nano-TiO₂, although they have similar diameters [81]. The results of the study confirmed the author's hypothesis since Nano-Co, but not Nano-TiO₂ induced MMP-2 and -9 activity and mRNA expression. Furthermore, Nano-Co decreased mRNA expression of TIMP-2. These modifications involved oxidants signaling mechanisms since 1) Nano-Co, but not Nano-TiO₂, 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 and coworkers [82] showed no induction of MMP-2 and TIMP-2 mRNA 1 month after rat exposure to aerosolized TiO₂ MNPs. Therefore, it appears that TiO₂ MNPs does not induce MMP-2, and that induction by Co MNPs involves oxidants-mediated signaling.

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 tire compounds, 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 [83]. However, despite their widespread use in industry, their toxicities and toxic mechanisms are not well understood. Choi and coworkers [84] investigated the degree of pulmonary fibrosis and the expression of fibrogenic mediators, including MMPs, in mice exposed to a suspension of ultrafine amorphous silica intratracheally 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 were significantly elevated at 24 h and 1 week post-instillation, though 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 in the expression of inflammatory cytokines IL-4, IL-10, IL-13 and IFN- 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 evaluated the respiratory effects of mixtures of different pollutants, somehow representing more closely a real life exposure than exposure to individual pollutants.

In order to investigate the mechanisms of cardiovascular diseases exacerbations induced by traffic related pollutants, Lund and colleagues [85] examined modulation of vascular MMP-2 and MMP-9 expression and activity by gasoline engine exhaust (GEE) in a well-characterized model of vascular toxicity, the apolipoprotein E knockout (ApoE $^{-/-}$) mouse. Animals were exposed to GEE (60 $\mu\text{g}/\text{m}^3$ particulate matter whole exhaust) or filtered-air for 6 hours per day 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 GEE, followed by gene induction of over the following week, leading to de novo synthesis of additional proteins and prolonged maintenance of vascular MMP response. These last findings were associated with vascular oxidative stress and ET-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 plasma of exposed mice. Interestingly, no markers of oxidative stress were detected in lung of exposed animals thereby indicating that the observed vascular effects of GEE may be independent of pulmonary oxidative stress pathways. The clinical relevance of these animal data was analysed in humans exposed for 2 h to a diesel engine exhaust. These subjects displayed a significant increase in plasmatic levels of MMP-9, ET-1 and NO $_x$ after exposure to diesel exhaust as 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 MMPs induction and activation, the same group analysed the comparative effects of various gases representative of gasoline exhaust on vascular toxicity and MMP-9 expression and activity [86]. Exposure atmospheres included gasoline and diesel engine exhaust, hardwood smoke, a simulated “downwind” coal combustion atmosphere (SDCCA), biogenically derived secondary organic aerosols (SOAs), and individual combustion source gases [nitric oxide (NO), nitrogen dioxide (NO₂), carbon monoxide (CO)]. The authors used the apolipoprotein E knockout (ApoE^{-/-}) mouse to assess comparative responses to these atmospheres. The animals were exposed 6h per day during 7 days. Eighteen hours after the end of exposure, the aorta and blood were sampled for analysis.

The diesel exhaust, SDCCA, hardwood smoke and SOA exposures were conducted at matching PM concentrations (300 µg/m³ for the present study). Because gasoline exhaust contains a very low mass of PM, mice were also exposed to a highest concentration used previously by the authors (60 µg PM/m³).

The main results of the study indicate that gasoline and to a lesser extent diesel exposure induced MMP-9 mRNA expression and activity in the aorta. Interestingly whereas gasoline exposure induced also an increased gelatinase activity, such 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 2 studies analyzed the consequences of MMP-9 activation in terms of its potential role in destabilizing vulnerable atherosclerotic plaques progression [87], which may be a predisposing factor for acute myocardial infarction. Furthermore, elevated plasma MMP-9 has been identified as a predictor of cardiovascular mortality

[88].

Also regarding the effects of combined gases and particles on MMPs, other group of investigators examined if MMPs are involved in woodsmoke-induced pulmonary emphysema. Indeed, COPD secondary to 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 and coworkers [89] develop a sequential model of subchronic exposure to woodsmoke in guinea pigs (60 g/day of pine wood, 5 days/week, from 1 to 7 months) and analyzed histological features, elastolysis, collagenolysis, gelatinolysis, and expression of MMP-1, -2, and -9. Histological analysis after 4 to 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 localized in epithelial cells and macrophages in wood exposed animals, along with expression of MMP-1. Collectively these results are close to those induced by cigarette smoke exposure in guinea pigs [90] and suggest a role of MMPs-1, -2, -9 and probably -12 in woodsmoke-induced pulmonary emphysema.

Discussion

Mechanisms and consequences of MMPs modulation by air pollutants

Most of the studies focused on MMPs and air pollutants examined their effects on MMP-2 and -9, some of them MMP-1 and only a few on MMP-12, the other MMPs were not or extremely few studied (see Table 1 for an overview). These studies analyzed constitutes a heterogeneous group of work when regarded in relation to the mechanisms of MMPs induction and their implications in the effects of air pollutants. Only a small number of publications were specifically dedicated to investigate 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 remodeling, carcinogenesis) than as specific study objects.

The summary of the main mechanistic data about MMPs induction by pollutants is the following:

i) MMP-2 and MMP-9 can be induced *via* TLR4 [91] (nickel exposure) or secondary to oxidative signaling [85] (gasoline exposure). In addition, MMP-9 can be induced *via* *fra-1*, a heterodimeric partner of activator protein (AP-1), which bind to and activates MMP-9 promoter (DEPs exposure)

ii) MMP-1 mRNA and protein expression can be induced *via* the MAP kinase ERK_{1/2}, secondary to the action of NOX4 or beta-arrestin (DEPs exposure), and

iii) Active MMP-12 is located in macrophages 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 reported and similar findings in different cell types, such astrocytes [92], human aortic smooth muscle cells [93], and synovial membrane cultures [94] after different kind of stimuli. 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 been also reported to be secondary to TLR4 activation after cigarette smoke exposure *via* a MyD88/IRAK1 [95] pathway. 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. NADPH Oxidase/NOX2 has been reported to mediate MMP-9 mRNA induction *via* reactive oxygen species production and ERK_{1/2} activation in macrophages [96]. NADPH oxidases/NOX 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 show that data generated in the field of MMPs modulation by air pollutants provide some interesting information for better understanding MMPs

biology. In general, these data were found in other models. Moreover, the amount of mechanistic information concerning MMPs 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 MMPs induction and/or activation, very few publications 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 previously, this study found an enhanced ozone-induced injury in MMP-9 $-/-$ mice, which was related to a difference in posttranscriptional 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 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 MMPs 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 of Li and coworkers [60] showing that a polymorphism of MMP-1 promoter predisposes to an enhanced gene transcription after DEP exposure. Whether this phenomena 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 show that exposure to carbon black nanoparticles enhances MMP-12 mRNA and protein expression in alveolar macrophages in a murin model of elastase-induced emphysema. The clinical relevance of this finding deserves further investigations. A role of MMPs in aggravating pulmonary diseases especially 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, lung cancer progression, among others [25, 26]) the clinical consequences of their induction in terms of mechanisms of development or aggravation of these diseases is very likely. However,

the formal proofs are still lacking.

Perspectives

Exposure to air pollutants has been associated to deleterious health effects for quite a long time. As for now, the implication of MMPs in these effects has been studied only for some pollutants, and a restricted amount of MMPs, while the link between MMP induction/activation and health effects is still scarce. A larger amount of studies is therefore deeply needed to better understand the implication of MMPs in health effects associated to air pollution. Moreover, the appearance of new pollutants, such as MNP, should deserve specific studies aimed to evaluate the role of MMPs in their potential health effects.

Acknowledgements

Maylis Dagouassat was supported by a grant of DIM SENT (Région Ile-de-France) and by the CARESEN foundation, Jorge Boczkowski by INSERM and Assistance Publique-Hôpitaux de Paris (Contrat Hospitalier de Recherche Translationnelle), and Sophie Lanone by INSERM and Centre Hospitalier Intercommunal de Créteil (Contrat Hospitalier de Recherche Translationnelle).

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