

REVIEW

Insights in particulate matter-induced allergic airway inflammation: Focus on the epithelium

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Summary

Outdoor air pollution is a major environmental health problem throughout the world. In particular, exposure to particulate matter (PM) has been associated with the development and exacerbation of several respiratory diseases, including asthma. Although the adverse health effects of PM have been demonstrated for many years, the underlying mechanisms have not been fully identified. In this review, we focus on the role of the lung epithelium and specifically highlight multiple cytokines in PM-induced respiratory responses. We describe the available literature on the topic including *in vitro* studies, findings in humans (ie observations in human cohorts, human controlled exposure and *ex vivo* studies) and *in vivo* animal studies. In brief, it has been shown that exposure to PM modulates the airway epithelium and promotes the production of several cytokines, including IL-1, IL-6, IL-8, IL-25, IL-33, TNF- α , TSLP and GM-CSF. Further, we propose that PM-induced type 2-promoting cytokines are important mediators in the acute and aggravating effects of PM on airway inflammation. Targeting these cytokines could therefore be a new approach in the treatment of asthma.

KEYWORDS

Asthma, Allergens, Air pollution, Particulate matter, Airway epithelium, Epithelial cytokines

1 | INTRODUCTION

Asthma is one of the most common chronic diseases worldwide, resulting in a serious global burden affecting as many as 334 million people.^{1,2} It is a chronic disease of the conducting airways characterized by a reversible airway obstruction, chronic airway inflammation, goblet cell metaplasia, airway hyperresponsiveness (AHR) and remodelling. Asthmatics suffer from symptomatic episodes of wheezing, coughing, breathlessness and chest tightness that vary over time and intensity.¹

Asthma is a complex and heterogeneous disease with different underlying disease processes. Multiple phenotypes can be distinguished based on the patient's clinical characteristics and inflammatory profile. The best characterized phenotype is (early-onset) allergic asthma, defined by the presence of allergen-specific immunoglobulin E (IgE) in serum and/or a positive skin prick test to common allergens, such as house dust mite (HDM), in association

with type 2-mediated immune responses. Other phenotypes are, for instance, late-onset eosinophilic asthma and smoking-associated neutrophilic asthma.^{3,4} Despite the heterogeneous nature of asthma, an almost identical pathological phenotype can be observed, characterized by an impaired epithelial barrier with areas of epithelial damage, excessive mucus production and thickening of the airway smooth muscles. Moreover, an excessive airway inflammation can be observed, which is a multicellular process involving mainly dendritic cells (DC), eosinophils, neutrophils, CD4⁺ T lymphocytes and mast cells.^{5,6}

There are several factors that can influence the development and phenotype of asthma (Figure 1).⁷ Twin studies have shown that there is a considerable genetic component in asthma, indicating that individuals can be genetically predisposed to be protected or more susceptible from developing asthma.⁸ Moreover, genome-wide association studies (GWAS) have identified several genetic polymorphisms related to the pathogenesis of asthma, such as TSLP and

IL-33, implicating an important role for airway epithelial damage in the induction of airway inflammation.^{9,10} Despite the power of GWAS to identify disease-associated loci, a significant proportion of asthmatics do not carry the known asthma gene polymorphisms.¹¹ Furthermore, over the last century, there has been a strong increase in asthma which cannot be explained by genetics but rather by gene-by-environment interactions.² Epigenetic changes, for instance, including DNA methylation, histone modifications and non-coding RNAs, are considered important additional mechanisms in the development of asthma and are highly influenced by environmental exposures.¹²⁻¹⁴ Moreover, it has been clearly demonstrated that exposure to a wide variety of environmental factors, such as allergens, respiratory infections and pollutants, is also an important determinant in the onset and aggravation of asthma.²

2 | AIR POLLUTION

Exposure to outdoor ambient air pollution represents a high environmental risk to human health, leading to approximately 3 million deaths each year.¹⁵ Several epidemiological studies have demonstrated a positive correlation between air pollution and adverse health effects, ranging from respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD), to cardiovascular illness and all-cause mortality, even at pollutant concentrations below the current national standards.¹⁶⁻²⁰ This ambient pollution is a complex mixture of chemicals such as ozone, nitrogen dioxide (NO₂), sulphur dioxide (SO₂), biological substances and particulate matter (PM),

derived from natural processes as well as from human activity, such as industry, cigarette smoke and traffic. Although all components of air pollution are harmful for human health, severe effects have been attributed to ambient PM as they can carry a broad range of toxic substances into the respiratory tract.²¹ Ambient PM consists of solid and liquid particles that differ in their chemical composition and are categorized based on their aerodynamic diameter (ie PM₁₀, PM_{2.5} and PM_{0.1}). When inhaled, PM₁₀ (particles smaller than 10 µm) deposits mainly in the nose and large conducting airways, whereas the deposition of PM_{2.5} (particles < 2.5 µm) is located throughout the respiratory tract, particularly in the small airways and alveoli. The ultrafine PM (UFPM) fraction PM_{0.1} (particles < 0.1 µm) will further deposit in the pulmonary tissue and some will even translocate from the alveoli to the pulmonary circulation.¹⁶ A major component of traffic-related ambient PM is diesel exhaust particles (DEPs). These particles consist of a carbon core which can adsorb several organic compounds, such as polycyclic aromatic hydrocarbons (PAHs) and quinones. Additional, traces of sulphates, nitrates and metals can be observed. The majority of DEPs belong to the highly respirable fine or ultrafine PM fraction.^{22,23}

Importantly, several key characteristics of ambient PM such as particle size, surface area and chemical composition will determine the specific mechanisms of PM-induced toxicological effects and their ability to induce lung injury and inflammation.²⁴⁻²⁶ Emerging evidence has shown that, among different particles, especially UFPM has a great toxic potential due to the unique physical and chemical properties.²⁶⁻²⁸ When the particle size decreases, the relative surface area increases, allowing a greater proportion of compounds to be

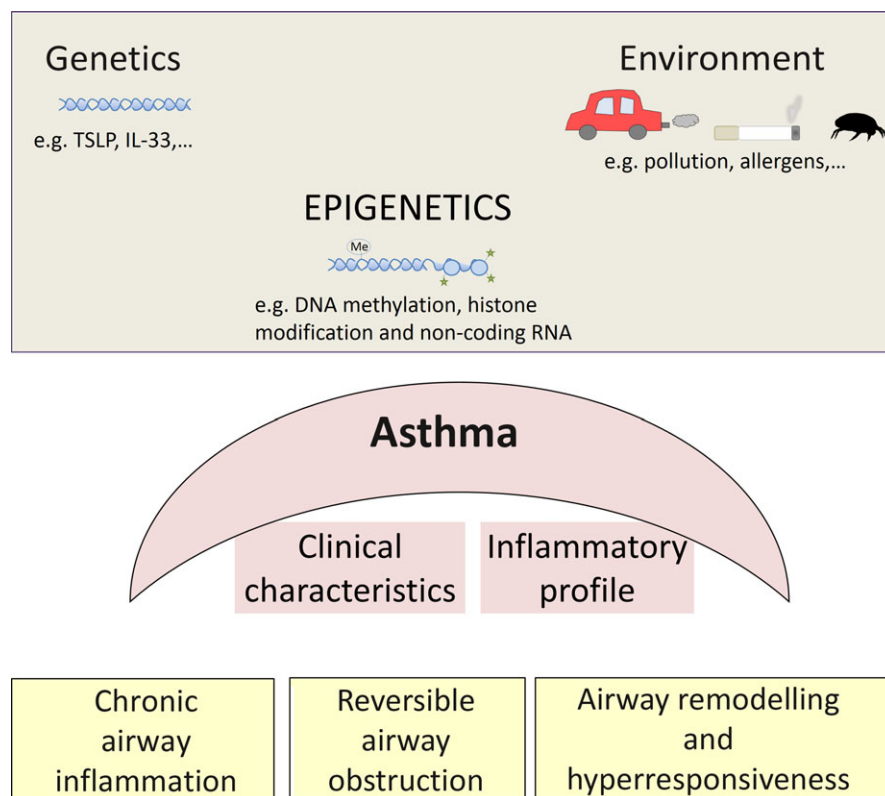


FIGURE 1 Risk factors contributing to the hallmarks of asthma. The development of asthma is strongly regulated by heritable components (genetics and epigenetic mechanisms) and exposure to environmental factors. Asthma is a heterogeneous disease wherein multiple phenotypes can be distinguished based on clinical characteristics and the inflammatory profile. The disease is being characterized by a chronic airway inflammation, reversible airway obstruction and airway wall remodelling and hyperresponsiveness

displayed at the surface.²⁴ UFPM contains significantly more metals and a large number of organic carbon compounds compared to larger particles,²⁹ which have been demonstrated to have pro-oxidant and pro-inflammatory effects in the respiratory system.²⁹⁻³¹ In addition, the high potency of UFPM to induce airway injury and inflammation is also due to a higher number of particles per mass unit, higher deposition efficiency and long-term retention/delayed clearance in the pulmonary region.^{32,33} Furthermore, several studies support the concept that UFPM is less likely to be phagocytized by alveolar macrophages and reside only a short period on the luminal side of the airway epithelium, penetrating deep into the tissue and cellular targets, lodge into the mitochondria, where cell injury, reactive oxygen species (ROS) generation and cell death can occur.^{34,35} Finally, the small size of UFPM facilitates its translocation from the respiratory epithelium towards the systemic circulation, causing extrapulmonary health effects.³³

3 | AIR POLLUTION AND THE AIRWAY EPITHELIUM

The airways are continuously exposed to particulate matter from the environment. Different mechanical filters located in the nasopharyngeal (ie nose hairs and the turbulence in the nasal turbinates), tracheobronchial and alveolar region protect the airways against inhaled foreign particles. Especially the mucociliary escalator is identified as a crucial self-clearing process of the airways to remove inhaled pathogens and particulates. More specifically, a large proportion of foreign particles are being trapped in the mucus layer on top of the airway epithelium and transported out of the airways by ciliary beating and coughing.^{36,37} Importantly, it was demonstrated that PM-exposed human bronchial epithelial cells (HBEC) progressively attenuate the ciliary beat frequency,³⁸ suggestive for a delayed clearance of these particles out of the lung. The particles that escape those defence mechanisms will then enter the lower airways and alveolar region where they can be endocytosed and removed by alveolar macrophages.^{36,37} Upon ambient PM exposure, these macrophages are activated and secrete an array of pro-inflammatory mediators, leading to apoptosis and induction of local and systemic inflammatory responses.³⁹ Interestingly, it was demonstrated that when exposed to ambient PM alveolar macrophages and airway epithelial cells can interact with each other to amplify the production of a variety of chemokines and cytokines, leading to inflammatory airway responses.⁴⁰

Initially, the airway epithelium was considered as a passive barrier which can exclude harmful agents from the underlying tissue by a dense network of tight and adherens junctions. In response to ambient PM exposure, this epithelial integrity can, however, be affected, as demonstrated by a down-regulation or disruption of the adhesion molecule E-cadherin or tight junction proteins, occludin and zonula occludens-1.⁴¹⁻⁴³ Nowadays, it is widely accepted that the epithelial layer also has an active role in the respiratory defence mechanisms, regulating both innate and adaptive immune responses.

First of all, several studies have shown that besides the specialized phagocytotic cells, such as the alveolar macrophages, ambient PM can also be endocytosed by airway epithelial cells.⁴⁴⁻⁴⁶ Although limited data are available concerning the exact underlying pathways, it is presumed that different uptake mechanisms can occur depending on the particle's characteristics.^{47,48} It was, for instance, demonstrated that the uptake of DEPs by lung epithelial cells was inhibited by cytochalasin D (CytoD), an inhibitor of actin-dependent uptake mechanisms, such as phagocytosis and micropinocytosis.⁴⁹ In contrast, uptake of UFPM was reported to be actin-independent.⁵⁰ Secondly, on the epithelial surface, different pattern recognition receptors (PRR) are expressed, which can be activated by pathogen-associated molecular patterns (PAMPs) in combination with the release of damage-associated molecular patterns (DAMPs)⁵¹⁻⁵³ (Figure 2). Indeed, it was already demonstrated that ambient PM can trigger toll-like receptor (TLR)2 and TLR4. Both receptors signal through MyD88, which will lead to the translocation of NF- κ B to the nucleus. Interestingly, depending on the particle's composition, distinct TLR may be activated. Until now, it is, however, unclear which components are responsible for TLR activation.⁵⁴⁻⁵⁷ Moreover, in addition to being a TLR ligand, ambient PM can also influence TLR expression and their ability to respond to other ligands, leading to altered inflammatory responses to subsequent stimuli.⁵⁶ Besides TLR, also cytosolic NOD-like receptors (NLR), such as NLRP3 (NOD-like receptor, pyrin domain containing 3), can be activated through environmental air pollutants and endogenous danger signals.⁵⁶ However, there is still some controversy regarding the role of the NLRP3 inflammasome upon pollutant exposure.^{58,59} Alternatively, ambient PM can also enhance the expression and activation of different membrane-associated receptors, such as the epidermal growth factor receptor (EGFR).⁶⁰⁻⁶⁵ Dimerization of the EGFR will stimulate tyrosine kinase activity that can elicit PI3K/AKT signalling and mitogen-activated protein kinases (MAPK) (ie ERK, JNK and p38), both involved in the activation of the transcription factor NF- κ B.^{30,63,66-69} Importantly, after internalization or PRR activation, exposure to ambient PM/DEPs will activate oxidative stress responses in the airway epithelial cells. When lung epithelial cells are exposed to ambient PM/DEPs higher levels of intracellular ROS formation, activation of oxidative stress-responsive genes, such as HMOX1, and oxidative damage has been demonstrated.⁷⁰⁻⁷⁴ Ambient PM/DEPs exposure was further associated with the overexpression of several genes and increased phosphorylated proteins related to the MAPK or PI3K/AKT pathway.^{30,66-68,70,74-76} Several studies also demonstrated that PM activates NF- κ B signalling in airway epithelial cells to induce inflammation.^{74,77-79}

4 | AIR POLLUTION AND ASTHMA

As stated above, DEPs can cause several adverse effects on their own, inducing acute inflammatory responses in the nose, throat and lungs.⁸⁰ Both human and animal studies have shown that ambient PM contributes to pulmonary inflammation through the induction of

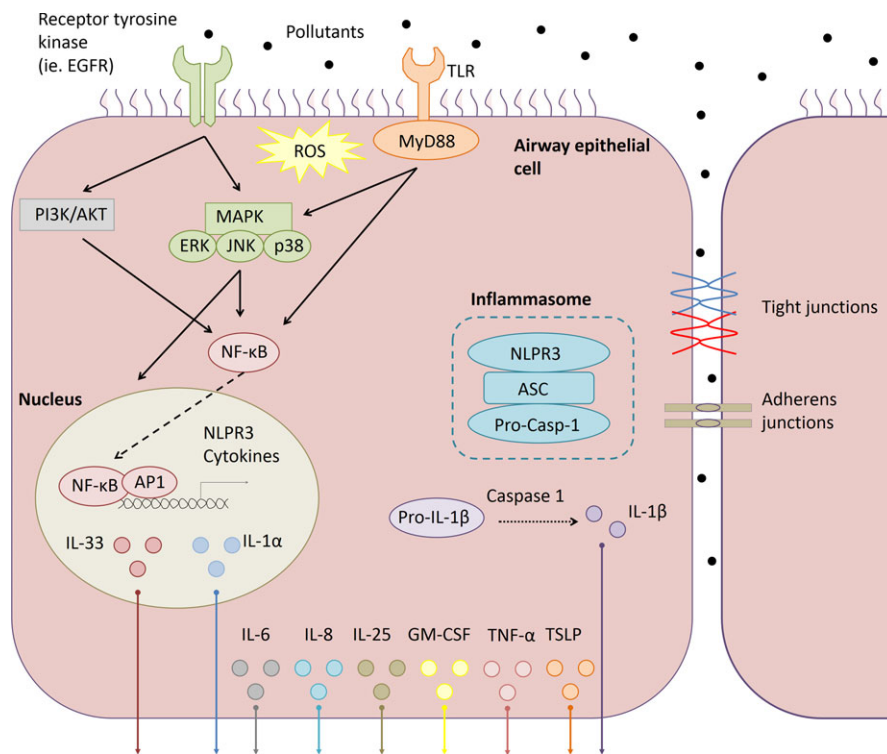


FIGURE 2 Mechanisms of PM-induced epithelial cytokine release. Exposure to PM induces production of ROS, epithelial disruption and activation of signalling pathways which are involved in the activation of NF-κB. Nuclear localization of NF-κB will then in turn lead to the transcription of NLRP3, involved in the NLRP3 inflammasome, and production of different cytokines. Both IL-33 and IL-1α are constitutively stored in the nucleus. Pro-IL-1β needs an additional cleavage to become functionally active. Conflicting results are obtained concerning the involvement of the NLRP3 inflammasome in the activation of pro-IL-1β. → activation; --- migration; — transcription; • secretion; — cleavage

oxidative stress, influencing cell signalling pathways and production of inflammatory mediators, leading to inflammatory cell recruitment.⁸¹⁻⁸⁴ Ambient PM inhalation also affects the autonomic nervous system (ie modulates heart rate variability⁸⁵) and induces bronchoconstriction and cough by sensory nerve activation.^{86,87} In addition, ambient PM inhalation initiates airway inflammation by activation of irritant receptors and acid-sensitive pathways located on sensory C nerve fibres lining the nasal and pulmonary airways. This results in the release of neuropeptides, like substance P (SP), leading to the initiation and maintenance of PM-mediated airway inflammation.⁸⁸ In both human asthmatics and rodent models, it has been suggested that exposure to DEPs augments neuropeptide-release through sensory nerve activation, leading to increased adverse effects of ambient PM on respiratory diseases.^{89,90}

Various epidemiological studies provided evidence that exposure to traffic-related air pollution can cause as well as exacerbate both childhood and adult-onset asthma.^{16,91-94} Living near major roads was, for instance, associated with significantly more respiratory symptoms and adverse effects on lung function in children and adults.^{18,95-98} Moreover, when asthmatic individuals were exposed to high levels of particulate pollution, they were more likely to experience worsened symptoms and impaired lung function, leading to uncontrolled asthma and hospitalizations.^{93,94,99} Controlled human exposure studies further confirmed that allergen-specific responses, such as airway eosinophilia, type 2 cytokine expression and allergen-specific IgE, can be augmented when the individuals were exposed to DEPs and an (neo)-allergen.¹⁰⁰⁻¹⁰²

Interestingly, the susceptibility towards the adverse effects of PM inhalation can be dependent on genetic factors.¹⁰³ Polymorphisms in

genes encoding for enzymes that regulate oxidative stress, *GSTM1*, *GSTP1* and *NQO1*, are identified as risk factors for allergic diseases in response to DEPs.¹⁰⁴ High DEPs exposure during infancy, for instance, could be related to an increased risk of wheezing among carriers with *GSTP1* polymorphism (*GSTP1* Val¹⁰⁵).¹⁰⁵ Furthermore, the aggravating effect of DEPs on allergic airway inflammation, that is IgE and histamine production, was enhanced when individuals had single nucleotide polymorphisms (SNPs) in the *GSTM1* and *GSTP1* gene.¹⁰⁶ Moreover, a *GSTM1* variant status led to decreased FEV₁ levels following DEPs and allergen co-exposure.¹⁰⁷ Finally, also genetic variants in the *TLR2* and *TLR4* genes influence the susceptibility to adverse effects of traffic-related ambient PM on childhood asthma.¹⁰⁸

By modulating various airway epithelial responses, including endocytotic uptake, ROS generation and PRR activation, it is suggested that pollutants can initiate or contribute to the pathological features of asthma.⁸⁰ Below, we will elaborate on the modulating effects of ambient PM, and in more detail of DEPs, on cytokine expression. In a first part, we will summarize the influence of ambient PM/DEPs on multiple cytokines in human airway epithelial cells in vitro. Secondly, we will highlight some type 2 mediated cytokines that have been shown to be involved in allergic asthma, including human studies and in vivo animal studies. The strengths and limitations^{80,109,110} of the different research approaches are summarized in Table 1. PubMed and Web of Science were searched using "particulate matter" or "diesel exhaust particles" in combination with "IL-1 OR IL-6 OR IL-8 OR IL-25 OR IL-33 OR GM-CSF OR TNF-α OR TSLP." A filter for research in the lung was applied. Additional relevant references were obtained from the reference list of retrieved manuscripts. Publications in English were considered until the 18th of January 2018.

TABLE 1 Advantages and limitations of basic and translational research to study the impact of PM on airway inflammation

	Advantages	Limitations
Epidemiological studies	Large cohorts "Real-world" exposure Mortality and morbidity end-points Study acute and chronic effects Study susceptible populations	Limited material (mostly blood) Estimation PM exposure Confounding factors/exposures Difficult to demonstrate causality
Controlled human exposure studies	Well-defined PM exposure Control confounding exposures Various end-points possible Intervention studies	Ethical issues Small subject numbers Exclusion of susceptible populations
In vitro studies	Investigate specific cells of interest Possibility of mechanistic and/or intervention studies	Different from the in vivo situation: artificial milieu with limited cell to cell interactions Difficult to mimic in vivo exposure
Rodent in vivo studies	Complete living organisms Mimic several asthmatic parameters Possibility of mechanistic and/or intervention studies Fully sequenced and highly homologue genome with humans Relatively easily housed, bred and handled	Ethical issues Differences in respiratory anatomy, physiology and immune system Difficult to mimic real-life exposure

4.1 | Particulate matter and epithelial cytokine expression: human in vitro studies

To evaluate the pro-inflammatory responses induced by ambient PM and DEPs in vitro, the epithelial gene expression and protein release can be measured (Table S1). When HBEC, derived from immortalized cell lines or normal donors, are exposed to ambient PM or DEPs, a strong up-regulation of different epithelial cytokines can be observed. The levels of IL-6,^{70,72,74,75,111-118} IL-8^{44,54,68,71,74-76,78,112,114,116-123} and TNF- α ,^{112,124} for instance, were significantly increased in cells that were exposed to PM. Of note, also MIP-3 α /CCL20,^{120,125} an important chemokine involved in DC recruitment, was increased in HBEC upon ambient PM exposure.

Over the years, a triad of epithelial-derived cytokines, interleukin (IL)-25, IL-33 and thymic stromal lymphopoietin (TSLP), have been identified as crucial regulators of various immune cells that contribute to the pathophysiology of asthma.¹²⁶⁻¹²⁸ In support of this, genome-wide association studies (GWAS) identified that polymorphisms in genes encoding for TSLP, IL-33 and its receptor IL1RL1 are associated with asthma risk.^{9,10} Several studies demonstrated

increased TSLP and IL-33 expression in the airways of asthmatics, which positively correlated with disease severity.¹²⁹⁻¹³¹ Moreover, in a subset of asthmatics, an increased epithelial expression of IL-25, which correlated with IL-25 plasma levels, could be demonstrated. Interestingly, no increase in IL-33 or TSLP was found in these patients,¹³² indicating the heterogeneity and complexity of the disease. In addition, IL-1 and granulocyte colony-stimulating factor (GM-CSF) have also been identified as important type 2-promoting cytokines⁵² and were both increased in the bronchial epithelium of asthmatics.^{133,134} Interestingly, these typical type 2-promoting cytokines, including, IL-1 α ,^{75,120} IL-1 β ,^{59,68,74,112,114,115,117,120,135} IL-33,¹³⁶ TSLP¹³⁷ and GM-CSF,^{44,67,112,119,120,138,139} were also increased in PM-exposed normal HBEC. In contrast, IL-25 levels were undetectable¹³⁹ or decreased.¹⁴⁰ Importantly, the number of studies regarding the modulating effects of PM on asthmatic HBEC is limited, and further research is needed. Considering that asthmatic epithelium is more susceptible towards oxidants that can affect apoptosis and epithelial damage, compared to normal airway epithelium,^{141,142} epithelial cells from asthmatics could be more sensitive towards the oxidative effects of PM. For the moment, this hypothesis is supported by the fact that elevated IL-33 production could be observed in PM-exposed asthmatic-derived epithelial cells, whereas this was not the case for normal HBEC that were exposed to PM.¹⁴⁰ Of note, as the IL-33 function can be modified by oxidation,¹⁴³ it could be that PM-generated ROS and oxidative stress could have important implications on IL-33 oxidation and subsequently its function. Furthermore, in asthmatic epithelium pro-inflammatory cytokines are constitutively more present and further increase upon low PM concentrations, whereas in normal HBEC, only the higher PM levels increased the release of IL-8 and GM-CSF.¹⁴⁴ Asthmatic ALI epithelial cell cultures also enhanced inflammatory responses towards PM, including increased IL-6, IL-8 and GM-CSF release, compared to non-asthmatic ALI cultures that were exposed to PM.¹⁴⁵ Further research where HBEC are exposed to PM in combination with allergens could be an interesting tool to investigate the aggravating effects of pollutants on allergen-induced epithelial responses.

Although it is clear that DEPs and PM can up-regulate the expression of numerous pro-inflammatory epithelial cytokines, not all studies reported an altered cytokine expression.^{70,72,75,139} Differences in epithelial responses can be explained by variations in chemical composition, more specifically by the amount of adsorbed organic compounds, metals and presence of endotoxins on the particle's surface. By extracting ambient PM or DEPs, for instance, it was demonstrated that especially the organic compounds were the main contributors to epithelial IL-1 β , IL-6, IL-8 and GM-CSF release.^{30,66,114,146,147} Moreover, employment of metal chelators led to a significant decrease in IL-1 α , IL-8 and GM-CSF expression, indicative for the contribution of metals in cytokine production.^{71,120} Removal of surface-bound endotoxins led to decreased cytokine responses, that is IL-1 α and GM-CSF,^{120,148} implicating endotoxins in the PM-induced epithelial cytokine release. In line, epithelial responses differed when sample collection was performed over

different time periods or locations.^{67,135,149,150} Furthermore, epithelial cytokine expression can be influenced by the particle's size,^{54,67,121,148,151} as well as the experimental set-up, the selected PM dose and epithelial cell culture.^{113,121,151-153}

5 | HOW DOES PARTICULATE MATTER INFLUENCE EPITHELIAL CYTOKINE EXPRESSION?

Mechanistic studies have already provided some insights on how pollutants modulate epithelial cytokine expression (Figure 2). Endocytotic uptake mechanisms or activation of PPR, for instance, are required for the induction of airway epithelial cytokine release upon PM exposure. Addition of CytoD, an inhibitor of actin-dependent uptake mechanisms, resulted in a significant reduction in PM-induced IL-8 and GM-CSF levels,^{66,71} whereas a blocking antibody to TLR2 inhibited the PM-induced IL-8 expression.⁵⁴ Moreover, DEPs-induced IL-8 and GM-CSF release were blocked by neutralizing antibodies towards EGFR or autocrine ligands of the EGFR, indicating that activation of the EGFR is an important pathway in DEPs-induced cytokine release.^{60,76,154} A crucial role for ROS formation during epithelial cytokine production was also identified, as different PM-induced cytokines, that is IL-1 α , IL-1 β , IL-6, IL-8 and GM-CSF, were significantly decreased after treatment with radical scavengers/antioxidants.^{30,63,66,71,74,113,120}

Exposure to pollutants can lead to the activation of several signalling pathways which will modulate cytokine gene expression. Upon DEPs exposure, the up-regulation of epithelial cytokines could be attributed to *de novo* protein and mRNA synthesis.^{66,119} Moreover, signal transduction via the MAPK or PI3K pathway was found to be crucial for cytokine production, as treatment with specific tyrosine kinases, MAPK or PI3K inhibitors led to a diminished release of IL-1 β , IL-6, IL-8 and GM-CSF in DEPs-exposed HBEC.^{30,63,66-68,74} Furthermore, using specific NF- κ B inhibitors or luciferase reporter gene assays, it was demonstrated that PM-induced NF- κ B activation controls IL-1 β , IL-6 and IL-8 production.^{74,78,113} Importantly, the production of ROS has been associated with the redox-sensitive transcription factor NF- κ B and the upstream signalling pathways. Pre-treatment with antioxidants significantly inhibited DEP-induced ERK/AKT phosphorylation, NF- κ B activation and subsequent cytokine production, that is IL-1 β , IL-6, IL-8 and GM-CSF.^{30,68,74,78,113} Overall, one can state that the PM-induced inflammatory responses, such as cytokine production, are predominantly mediated by the NF- κ B pathway which is activated by ROS and MAPK. Of note, dependent on the amount of organic compounds that are present on DEPs, a differential transcriptional regulation of IL-8 expression was proposed. Whereas exposure to DEPs with a lower organic content involved the NF- κ B pathways to induce IL-8 promoter activity, high organic DEPs were associated with an AP-1-dependent mechanism.¹⁵⁵

However, identifying additional upstream signalling pathways that regulate cytokine production in response to pollutants is of great interest for new intervention strategies. Recently, a novel

mechanism was proposed whereby the expression of TSLP was regulated by miRNA-375 via the aryl hydrocarbon receptor (Ahr). More specifically, it was suggested that the suppressive effects of Ahr on NF- κ B's transcriptional activity are down-regulated by miRNA-375, leading to increased TSLP expression.¹⁵⁶ Moreover, increased IL-6 and IL-8 production upon PM exposure was suggested to depend on a sustained activation of NF- κ B, which was regulated by a ROS/PI3K/AKT-dependent down-regulation of miRNA-331 expression in airway epithelial cells.⁷⁹ Notably, mechanistic insights concerning the altered expression of typical type 2-promoting cytokines, such as IL-25, IL-33 and TSLP, towards PM exposure are highly lacking.

5.1 | Particulate matter and type 2-promoting cytokines: expression and mechanisms in humans and murine in vivo models

Considering that in the pathogenesis of allergic asthma, typical type 2-promoting cytokines, such as IL-1, IL-25, IL-33, GM-CSF and TSLP, have been implicated, we will now further elucidate on their role using human studies and in vivo animal studies (Table S2 and S3 respectively).

By collecting data from PM measuring stations, estimating the proximity to major roads or performing controlled human exposure and ex vivo studies, associations between pollution exposure and inflammatory markers are being explored in humans. When IL-1 β or GM-CSF was measured locally, in exhaled breath, lavage fluid or bronchial tissue, no associations with PM could be observed.¹⁵⁷⁻¹⁵⁹ The effect of PM on local cytokine release is, however, an underexplored research field, with a limited number of studies available. In contrast, most human studies investigate the effect of air pollution using blood, in which the identified cytokines can have either an epithelial or hematopoietic origin. Notably, exposure to PM has generally been related to an increased expression of IL-1 β in the blood of adults, children and during prenatal life.¹⁶⁰⁻¹⁶⁷ One study also observed a correlation between a joint increase in TSLP+IL-33 cord blood levels and heavy street traffic during pregnancy¹⁶⁸ (see Table S2 for a detailed overview).

In vivo research in mice, that is using specific knockout strains and monoclonal antibodies, can provide additional insights into potential mechanisms how air pollution can modulate (allergic) airway inflammation and offer the opportunity to validate hypotheses that have been generated by human studies. First, during acute DEPs-induced inflammatory lung responses, an increased expression of IL-1 β ^{58,59,64,169-177} and GM-CSF^{139,178} in BALF and lung tissue were observed, whereas 1 study reported IL-25, IL-33 and TSLP as undetectable.¹³⁹ Moreover, the increased IL-1 β and GM-CSF levels could be linked to PM or DEPs-induced inflammation and AHR.^{58,175,178} Interestingly, using a mouse model of acute DEP-induced lung inflammation, IL-1 β /IL-1RI signalling was identified as a critical regulatory pathway of the inflammatory responses towards DEPs.⁵⁸ Some murine in vivo studies, however, did not demonstrate an up-regulation of IL-1 β ^{173,179} or GM-CSF^{169,180} after acute PM or DEPs exposure, possibly due to different exposure protocols, time

of sampling, murine strains and pollutant composition. Secondly, in murine models of DEPs-enhanced allergic airway inflammation, increased pulmonary levels of IL-1 α ¹⁸¹ and GM-CSF^{181,182} in OVA models and higher IL-25,¹⁸³ IL-33¹⁸³ and GM-CSF¹⁸⁴ levels in the HDM models were observed. In other studies, however, similar or decreased GM-CSF levels were found in DEPs+OVA-exposed mice compared to sole OVA exposure¹⁸⁵⁻¹⁹⁰ or were undetectable during DEPs+HDM administration¹⁹¹ (see Table S3 for a detailed overview). Notably, in these studies, the exact cellular origin of these cytokines was, however, not determined. Moreover, to date, most studies only focused on cytokine expression, whereas mechanistic studies to unravel the contribution of these type 2-promoting cytokines in DEPs-induced development and aggravation of allergic asthma are scarce.

Besides the release of typical type 2-promoting cytokines, also chemokines, like CCL2/MCP-1, CCL20/MIP-3 α and chemerin, are

released upon DEPs exposure, contributing to recruitment of DC towards the pulmonary tissue.^{81,192,193} DEPs are internalized by DC, that in response to the type 2-promoting cytokines will mature and migrate towards the MLN,^{81,136-138} which was found to occur in a CCR7-dependent manner.⁸¹ DEPs can thereby bind allergens onto their surface, acting as carriers to increase allergen deposition. Moreover, co-administration of allergens and pollutants can facilitate the penetration of allergens into the airway mucosa, resulting in a higher recruitment, uptake, maturation and migration of these DC.^{80,194} In the mediastinal lymph nodes, DEPs can affect the responses towards allergens by enhancing T_H2 cell polarization.^{81,136-138} In this manner, DEPs can increase the allergic responses, characterized by increased inflammatory cell influx, type 2 cytokine production, immunoglobulin production, mucus metaplasia and AHR^{80,183,192,195} (Figure 3). In addition to the modulating effects of DEPs on the DC-T_H2 pathway, also innate lymphoid cells type 2 (ILC2) can become activated by the

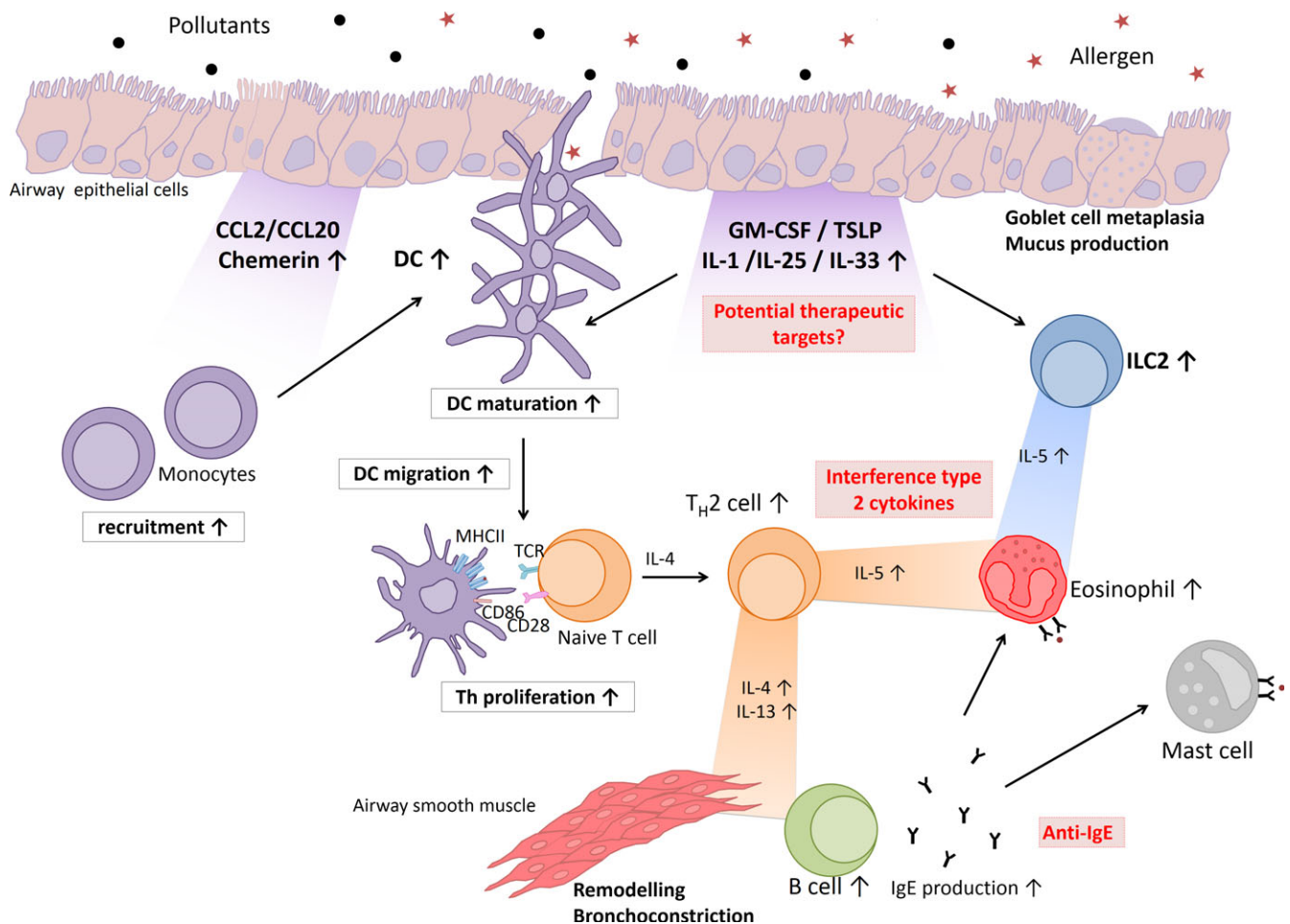


FIGURE 3 Diesel exhaust particles affect epithelial cytokines in the pathogenesis of allergic airway inflammation. Exposure to DEPs will activate the airway epithelium to secrete specific chemokines, leading to an increased recruitment of monocyte-derived DC to the pulmonary tissue. Specific epithelial-derived cytokines in combination with allergen and pollutant uptake will result in increased DC maturation, migration towards the MLN and induction of Th2 responses. The same epithelial-derived cytokines will also activate ILC2. These Th2 cells and ILC2 will then in turn secrete specific type 2 cytokines inducing airway eosinophilia, mucus metaplasia, airway wall remodelling and airway hyperresponsiveness. Cells and mediators that increase upon combined allergen and pollutant exposure are indicated with a black arrow. ●=pollutants, ★=allergen, CCL = C-C motive chemokine ligand, DC = dendritic cell, IgE = immunoglobulin E, IL = interleukin, ILC2 = innate lymphoid cell type 2, Th = T helper. Therapeutic targets are indicated in a red box

secretion of some specific type 2-promoting cytokines.^{196,197} Recently, it was, however, demonstrated that the aggravating effect of DEPs on allergic airway inflammation was predominantly dependent on the adaptive immune system, rather than ILC2.¹⁸³ In addition to the effects of pollutants on asthma development, murine exacerbations models can be used to evaluate the aggravating effects of PM or DEPs on allergen-induced airway inflammation.⁸⁰ Interference with anti-IL-33, for instance, suggested a role for IL-33 during an acute PM-induced exacerbation in a model of established chronic allergic asthma.¹⁹⁰

Despite many asthmatic features, including pulmonary inflammation, remodelling and AHR, that can be replicated in murine models, also important interspecies differences in immunology, respiratory physiology and anatomy should be taken into account.^{80,198} The expression of human IL-33, for instance, is mainly localized in bronchial epithelial cells, whereas in the mouse IL-33 is predominantly expressed by alveolar type II pneumocytes,¹⁹⁹ which may be important when investigating the effects of air pollution in *in vivo* models. Moreover, differences in particle distribution due to a distinct pulmonary lobulation, bronchial branching and altered clearance and filtering processes can affect the pulmonary responses towards PM exposure.⁸⁰ Caution is therefore advised when extrapolating results obtained from murine models to the human situation. Furthermore, some general differences between human and murine studies have been summarized in table 1.

6 | POTENTIAL THERAPEUTIC TARGETS IN THE TREATMENT OF POLLUTANT-INDUCED AIRWAY INFLAMMATION

In addition to standard therapy, poor-controlled, severe asthmatics with a specific asthmatic phenotype are treated with a few approved biologics, that is anti-IgE (omalizumab) or anti-IL-5 (mepolizumab or reslizumab)).²⁰⁰ Moreover, also several biologics that target the type 2-promoting cytokines, such as anti-TSLP (tezepelumab),²⁰¹ anti-GM-CSF²⁰² and anti-IL-33,²⁰³ have entered (pre)clinical trials to evaluate their efficacy as add-on therapy in asthmatics. Intriguingly, as severe asthmatic phenotypes are associated with exposure to air pollutants, microbes and glycolipids,²⁰⁴ this raises the hypothesis that these biologics would also be beneficial within the context of PM exposure. Furthermore, considering that exposure to air pollution can lead to the development of neutrophilic asthma³ and reducing IL-1 β levels diminished airway neutrophilia, targeting IL-1 signalling pathways could also be beneficial.²⁰⁵

Besides modifying the expression of type 2-promoting cytokines, implications of PM on allergic inflammation and asthma exacerbations are for a significant part due to the production of ROS.²⁰⁶ As pretreatment with antioxidants can affect intracellular signalling pathways and decrease cytokine production,^{30,68,74,78,113} enhancing the levels of the antioxidant superoxide dismutase (SOD) or influencing the glutathione (GSH) system, for instance, could be another approach to lower oxidative stress and airway inflammation.²⁰⁷⁻²¹⁰

Especially since both asthma and PM exposure have already been associated with a significant reduction in SOD or polymorphisms in genes implicated in the GSH system have been detected.^{104,106,211-213} Moreover, also dietary antioxidants,^{209,214} such as selenium, were already associated with reduced oxidative stress and lung inflammation in a model of PM-induced lung injury.¹⁷⁷ Furthermore, selenium supplementation improved symptoms in asthmatics compared to placebo.²¹⁵

7 | CONCLUSIONS AND FUTURE PERSPECTIVES

Although PM is without a doubt associated with an increased risk of asthma development and exacerbations, specific mechanisms are incompletely known. Epidemiological studies, as well as the use of both *in vitro* and *in vivo* models, have revealed a central role for the airway epithelium and type 2-promoting cytokines during pollutant exposure. The modulating effects of particulate matter on multiple cytokines can therefore be proposed as an important driver of allergen-induced airway responses. It is, however, crucial that the interaction of multiple environmental factors and the mechanisms involved are further elucidated. Additional research on the role of type 2-promoting cytokines during pollutant-enhanced airway inflammation may lead to the discovery of better therapeutics in the treatment of asthma.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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SUPPLEMENTAL DATA

TABLE S1: PARTICULATE MATTER AND EPITHELIAL CYTOKINE EXPRESSION: HUMAN <i>IN VITRO</i> STUDIES				
Material		Exposure protocol	Observations epithelial cytokines	Reference
IMMORTALIZED CELL LINE	16HBE14o- cells	DEPs (SRM 1650) and CB (carbon black) at 10 µg/cm ² for 6- 48h	Time-dependent DEPs-induced ↑ IL-8 and GM-CSF secretion. CB ineffective.	Boland et al., 1999
	BEAS-2B cell line	0.1-100 µg/mL DEPs for 24h	Time and dose dependent ↑ IL-8 mRNA expression upon DEPs exposure Involvement ROS and NF-κB	Takizawa et al., 1999
	16HBE14o- cells	2.5-20 µg/cm ² DEPs (SRM 1650) for 6-48h	Time and dose dependent ↑ GM-CSF release in the presence of DEPs Importance organic compounds (lower effect of extracted DEPs and CB ineffective), ROS, tyrosine kinases and <i>de novo</i> protein synthesis	Boland et al., 2000
	16HBE14o- cells	10 µg/cm ² DEPs, organic extracts of DEPs, stripped DEPs (SRM 1650) or carbon black for 24h	↑ GM-CSF secretion by DEPs and organic extracts of DEPs. Importance organic compounds (lower effect of stripped DEP and no effect of CB), involvement of ROS and MAPK pathways	Bonvallot et al., 2001
	BEAS-2B cell line	100 µg/mL PM at different locations for 4-16h	↑ IL-6 release by all PM samples	Veronesi et al., 2002
	16HBE14o- cells	DEPs (SRM 1650), RPM (SRM 1648), atmospheric PM _{2.5}	Dose dependent ↑ GM-CSF release after DEP/PM exposure. DEPs had a higher effect compared to PM.	Baulig et al.,

		and CB at 10-30 $\mu\text{g}/\text{cm}^2$ for 24h	CB are ineffective.	2003
	16HBE14o- cells	10 $\mu\text{g}/\text{cm}^2$ multiple $\text{PM}_{2.5}$ samples/DEPs for 24h	$\uparrow$ GM-CSF and TNF- α release for all PM/DEPs samples	Baulig et al., 2004
	16HBE14o- cells	$\text{PM}_{2.5}$ and DEPs (SRM1650) at 10 $\mu\text{g}/\text{cm}^2$ for 24h	Causal relationship between amphiregulin and GM-CSF production in response to DEPs and PM	Blanchet et al., 2004
	L132 cell line	$\text{PM}_{2.5}$ at 18-75 $\mu\text{g}/\text{mL}$ for 24-72h	Time and dose dependent $\text{PM}_{2.5}$ -induced $\uparrow$ IL-1 β , IL-6, IL-8, TNF- α release and IL-1 β , IL-6, IL-8, TNF- α and GM-CSF mRNA expression	Dagher et al., 2005
	16HBE14o- cells	$\text{PM}_{2.5}$ at 10 $\mu\text{g}/\text{cm}^2$ for 24h	$\uparrow$ GM-CSF protein levels after PM exposure EGFR ligands involved in GM-CSF release	Rumelhard et al., 2007
	16HBE14o- cells	Coarse, intermediate, fine and ultrafine PM for 24h at 1 or 10 $\mu\text{g}/\text{cm}^2$	Dose dependent $\uparrow$ GM-CSF release following PM exposure. GM-CSF levels decreased with increasing aerosol size Role for endotoxins	Ramgolam et al., 2009
	16HBE14o- cells	DEPs (SRM1650), CB or $\text{PM}_{2.5}$ samples at 10 $\mu\text{g}/\text{cm}^2$ for 24h	$\uparrow$ GM-CSF protein after treatment with DEPs and $\text{PM}_{2.5}$ from all sites. CB are ineffective ROS induction, EGFR and MAP kinases signalling pathways involved in the GM-CSF release.	Baulig et al., 2009
	BEAS-2B cell line	1.25-20 $\mu\text{g}/\text{cm}^2$ coarse and fine PM or 5-80 $\mu\text{g}/\text{cm}^2$ SRM1648 for 24h	Dose-dependent $\uparrow$ IL-8 release following coarse PM and SRM1648. No response towards fine PM	Ovrevik et al., 2009
	BEAS-2B cell line	Organic $\text{PM}_{2.5}$ at 1-100 $\mu\text{g}/\text{mL}$ for 24h	Polar organic extracts induce dose-dependent $\uparrow$ IL-1 β secretion	Fuentes-Mattei et al., 2010

	BEAS-2B cell line	10 µg/cm ² DEPs with varying organic content for 4h	↑ IL-8 mRNA expression with DEPs exposure to low and high organic content	Tal et al., 2010
	BEAS-2B cell line	Different PM samples at 3-12 µg/cm ² for 24-72h	Dose-dependent ↑ IL-1β, IL-6 and IL-8 mRNA levels for all different PM samples. No differences in TNF-α mRNA ↑ IL-6, IL-8 and TNF-α protein levels in the three PM samples. No difference in IL-1β protein levels	Dieme et al., 2012
	16HBE14o- cells	PM _{2.5} at 0.1-10 µg/cm ² for 2-48h	Time-dependent PM _{2.5} -induced GM-CSF ↑ at both mRNA and protein level GM-CSF release dependent on autocrine effect of EGFR ligands	Ramgolam et al., 2012
	BEAS-2B cell line	12.5 or 25 µg/mL PM _{2.5} or PM ₁₀ 6-24h	PM _{2.5} -induced ↑ IL-1α and IL-6 release, ↑IL-8 only after 24h IL-1β was undetectable	Watterson et al., 2012
	BEAS-2B cell line	100 µg/mL DEPs for 4h	↑ IL-1β, IL-6 and IL-8 mRNA and protein levels when exposed to DEPs	Totlandsdal et al., 2012
	BEAS-2B cell line	100 µg/mL DEP for 2,4 or 6h	↑ IL-6 and IL-8 mRNA expression after DEP exposure	Bach et al., 2014
	HBEC (HBEC-6KT)	500 µg/mL PM ₁₀ (EHC-93) for 24h	PM ₁₀ -induced ↑ IL-1β and GM-CSF production. IL-1β release proceeds GM-CSF production. No detection of TSLP, IL-25 and IL-33 NLRP3 inflammasome-dependent	Hirota et al., 2015
	16HBE cells	PM _{2.5} at 25-150 µg/mL for 5 and 24h	Dose and time-dependent ↑ IL-6 production Decrease in IL-8 and TNF-α	Zhou et al., 2015
	BEAS-2B cell line	100 µg/mL PM _{2.5} from six cities of China for 24h	↑ IL-1β mRNA levels by all PM _{2.5} samples	Yang et al.,

			Association with concentration of metals (Ni) on PM	2016
	BEAS-2B cell line	2.5-10 $\mu\text{g}/\text{cm}^2$ PM _{2.5} and 10 $\mu\text{g}/\text{cm}^2$ DEPs for 24h	Dose dependent $\uparrow$ IL-8 release after PM/DEPs exposure Involvement ROS and endocytosis Importance metals	Yan et al., 2016
	BEAS-2B cell line	100 $\mu\text{g}/\text{mL}$ PM ₁₀ from three different locations	$\uparrow$ IL-8 release by all PM samples	Van Den Heuvel et al., 2016
	BEAS-2B cell line	2 $\mu\text{g}/\text{cm}^2$ PM _{2.5} for 72h	$\uparrow$ IL-6 and IL-8 production following PM exposure	Leclercq et al., 2017
	BEAS-2B cell line	5 $\mu\text{g}/\text{cm}^2$ diesel UFP for 20h	$\uparrow$ IL-6 release after DEP No difference in IL-8 and TNF- α Importance ROS and MAPK related genes	Bengalli et al., 2017
HUMAN BRONCHIAL EPITHELIAL CELLS (HBEC)	HBEC from normal bronchus	10-100 $\mu\text{g}/\text{mL}$ DEPs or 2.5-2500 $\mu\text{g}/\text{mL}$ suspended PM for 2-48h	Dose dependent $\uparrow$ IL-8 and GM-CSF release by DEPs exposure <i>De novo</i> protein synthesis	Ohtoshi et al., 1998
NORMAL SUBJECTS	HBEC from bronchial brush biopsies normal subjects	Ultrafine, fine, intermediate and coarse PM at 25-100 $\mu\text{g}/\text{mL}$ for 18h	Dose dependent $\uparrow$ GM-CSF release to ultrafine/fine PM. No dose-response was detected for the larger PM fractions or carbon particles of the fine sizes. Involvement of MAPK-signaling cascade	Reibman et al., 2002
	HBEC from airway biopsy	50-250 $\mu\text{g}/\text{mL}$ coarse, fine and ultrafine PM for 24h	PM-induced $\uparrow$ IL-8 release Involvement of TLR2	Becker et al., 2005
	HBEC from lung	Baltimore 10-100 $\mu\text{g}/\text{mL}$ PM	Dose and time-dependent $\uparrow$ IL-6 release to PM	Zhao et al.,

	transplant donors	for 1-24h	Involvement of ROS and NF-κB pathway	2008
	HBEC (Lonza)	10 µg/mL PM for 3h	PM-treated HBEC ↑ IL-1α, IL-1β, IL-8 and GM-CSF mRNA levels Role for endotoxin on the particles and ROS production	Wong et al., 2011
	HBEC from brush biopsy	20-80 µg/cm ² PM ₁₀ and PM _{2.5} for 4h	Dose-dependent ↑ IL-8 mRNA levels to each particle type Similar responses in BEAS-2B cell line	Silbajoris et al., 2011
	HBEC by brush biopsy	25-100 µg/ mL DEPs for 24h	Dose-dependent ↑ IL-1β and IL-8 protein expression by DEPs exposure Involvement glutathione S-transferases (GSTs) through ROS-associated ERK and Akt activation.	Wu et al., 2012
	HBEC from 5 healthy subjects	500 µg/mL PM ₁₀ (EHC-93) for 24h	↑ IL-1β production by PM ₁₀ exposure Involvement NLRP3 inflammasome	Hirota et al., 2012
	HBEC (Lonza)	3 µg/cm ² DEPs and fine PM for 6, 18h	Dose dependent DEPs-induced ↑ TSLP mRNA miRNA-375 involved in the TSLP regulation	Bleck et al., 2013
	Air-liquid interface (ALI) culture NHBE (Lonza)	4 treatments of 4h (spaced 48h) with DEPs (SRM1650; 2, 5 and 10 µg/cm ²) or coarse / fine / ultrafine PM (1, 5 and 10 µg/cm ²)	↑ GM-CSF release after each DEPs/PM treatment that could be maintained up to 5 weeks post exposure. PM had a lower effect than DEPs ↑ IL-6 release after DEPs/PM treatment that could be maintained up to 5 weeks post exposure ↑ IL-1β release at the beginning of the PM treatment	Boublil et al., 2013
	HBEC from bronchial brushings (submerged / ALI)	Coarse, fine and ultrafine PM at 50-250 µg for 4h	↑ IL-6 and IL-8 in submerged PM-exposed HBEC No response in ALI cultures	Ghio et al., 2013

	HBEC from bronchial brushings	10-200 µg/mL DEPs for 18-24h	DEPs-induced dose-dependent ↑ IL-8 mRNA and protein level Involvement of EGFR/autocrine ligands and MAPK pathway	Parnia et al., 2014
	HBEC from brushings	1.1 – 11.1 µg/cm ² coarse, fine and ultrafine PM for 24h	Dose-dependent PM-induced ↑IL-8 release Importance particle size and epithelial cell culture	Loxham et al., 2015
	HBEC of bronchial brushings	50 µg/mL PM ₁₀ or CB for 24h	PM ₁₀ stimulated ↑ IL-1β, IL-6 and IL-8 mRNA expression. CB is ineffective.	Kumar et al., 2015
	HBEC	100-500 µg/cm ³ Urban PM 1649b (NIST) for 24h	Dose-dependent ↑ IL-1β, IL-6 and IL-8 at both mRNA and protein level after PM exposure Involvement of ROS, MAPK pathway and NF-κB	Wang et al., 2017
HBEC ASTHMATICS	HBEC from asthmatic and non-asthmatic subjects	0-100 µg/mL DEPs for 24h	HBEC of asthmatics constitutively ↑ GM-CSF secretion compared to non-asthmatics + DEPs: ↑↑ GM-CSF release in asthmatics	Devalia et al., 1999
	ALI cultures asthmatic and non-asthmatics	100 µg/mL PM (EHC-93) for 24-96h	PM-exposed HBEC from asthmatics have an enhanced ↑ IL-6, IL-8 and GM-CSF release compared to non-asthmatics	Hackett et al., 2011
CO-CULTURE	mDC from blood / HBEC from bronchial brushing normal volunteers	10µg/mL DEPs for 48h	↑ GM-CSF release in the presence of DEP-treated HBEC GM-CSF-induced DC maturation	Bleck et al., 2006

	mDC from blood / HBEC from bronchial brushing normal volunteers	3 µg/cm ² DEPs for 4- 18h	↑ TSLP mRNA and protein expression in DEPs-exposed HBEC TSLP induced maturation and T cell proliferation of DC	Bleck et al., 2008
	HBEC mDC from blood	3 µg/cm ² DEPs for 6- 48h	DEP-treated HBEC ↑ IL-33 transcription and protein levels IL-33 induced functional mDC maturation	Bleck et al., 2011
	Co-culture HBEC of bronchial biopsies and blood moDC from control and asthmatic subjects (ALI-culture)	PM for 24h	Monoculture HBEC + PM exposure: ↑ IL-33 production in HBEC from severe asthmatics PM-induced ↓ IL-25 mRNA in control subjects No effect on TSLP Co-culture HBEC + moDC + PM No effect on TSLP, IL-33 or IL-25 (PM impaired TSLP and IL-33 levels that was increased in co-culture of severe asthmatic HBEC)	Gras et al., 2017

↑: cytokine increases in response to DEPs/PM, ↓: cytokine decreases in response to DEPs/PM, ≈: equal cytokine levels

TABLE S2: PARTICULATE MATTER AND TYPE-2 PROMOTING CYTOKINES: FINDINGS IN HUMANS				
Study population		Material	Observations epithelial cytokines	Reference
ADULT	15 healthy volunteers exposed to diluted DE and air for 1h	Bronchial tissue Bronchial wash cells	≈ IL-1β and GM-CSF gene transcript levels after DE exposure compared with air	Salvi et al., 2000
	Intranasal instillation 300μg NIST 1650 DEP both asthmatic and non-asthmatic subjects	Nasal lavage	≈ GM-CSF protein levels post DEP exposure	Kongerud et al., 2006
	87 healthy subjects (office workers vs. traffic-controllers) Monitoring PM ₁₀	Exhaled breath condensate (EBC) Nasal lavage fluid (NLF)	≈ IL-1β protein levels in both NLF and EBC between traffic-controllers and office workers	Lima et al., 2013
	Colaui study Monitoring PM ₁₀ on the day of visit	Blood	Positive association ↑ IL-1β protein levels for every 10μg/m ³ elevation in PM ₁₀	Tsai et al., 2012
	40 subjects (20 near highway and 20 urban residents)	Blood	Positive association ↑ IL-1 protein levels with proximity to highways or heavy traffic	Brugge et al., 2013
	15 healthy subjects exposed to DE and filtered air for 2h	Blood	↑ IL-1β protein levels post DE exposure	Krishnan et al., 2013
	23 healthy adults walking 2 hours along three diverse roadways Mobile PM sampling	Blood	Positive association ↑ IL-1β protein levels with increasing concentrations PM _{2.5}	Mirowsky et al., 2015

	72 healthy subjects Monitoring PM _{2.5}	Blood	Positive association ↑ IL-1β protein levels with a 10μg/m ³ increase in PM _{2.5}	Pope et al., 2016
	85 male subjects (45 taxi drivers vs. 40 office workers)	Blood	↑ IL-1β protein levels in taxi drivers compared to the control group	Barth et al., 2017
PRENATAL LIFE	Birth cohort Monitoring maternal exposure to PM ₁₀ in the third trimester Proxy traffic-related air pollution (distance to major roads)	Cord blood	Positive association ↑ IL-1β protein levels with PM ₁₀ GM-CSF expressed only in 2% of samples No strong associations between major road distance and cytokine concentrations	Latzin et al., 2011
	MIREC study Self-reported exposure to street traffic	Cord blood	Positive association Jointly ↑ TSLP and IL-33 protein levels with heavy street traffic	Ashley-Martin et al., 2015
	MIREC study Monitoring Maternal exposure to PM _{2.5}	Cord blood	No association of TSLP and IL-33 protein levels with PM _{2.5}	Ashley-Martin et al., 2016
CHILDREN	Residential distance to a major road of 577 control and asthmatic children	Blood	↑ IL-1β or IL-33 protein levels in children with asthma compared to controls. No associations of IL-1β or IL-33 protein levels with residential distance to a major road	Rosser et al., 2016
	27 children with asthma and 12 controls (without asthma)	Blood	↑ IL-1β release after ex-vivo stimulation with traffic-related particulate matter for 24h No differences between asthmatics and controls	Negherbon et al., 2017

↑: cytokine increases in response to DEPs/PM, ↓: cytokine decreases in response to DEPs/PM, ≈: equal cytokine levels

TABLE S3: PARTICULATE MATTER AND TYPE-2 PROMOTING CYTOKINES: <i>IN VIVO</i> ANIMAL MODELS					
Animal		Exposure protocol	Observations epithelial cytokines	Reference	
Acute effects of DEPs/PM	Male A/J and C57BL/6 mice (10-12 weeks)	Intranasal administration 0.25mg/mL DEP in 40µL every other day for 2 weeks	DEP-stimulated ↑ GM-CSF mRNA total lung tissue Role for GM-CSF in AHR, BALF cell recruitment and mucus metaplasia	Ohta et al., 1999	
	Sprague-Dawley rats	Intratracheal administration 5-50 mg DEPS (NIST)	↑ IL-1β mRNA in BALF cells day 1 and 30 post exposure with high DEPs levels ≈ GM-CSF mRNA lung tissue	Rao et al., 2005	
	Male ICR mice (6 weeks)	Intratracheal administration 500µg DEPs	DEP-induced ↑ IL-1β protein levels in lung tissue supernatants compared to vehicle	Inoue et al., 2006	
	Female BALB/c mice (10-12 weeks)	Intratracheal administration 5-50µg DEPs	≈ BALF IL-1β levels in DEPs and control-exposed groups	Stoeger et al., 2006	
	rats	Intratracheal administration 1-5 mg DEPs on day 1	No detection of GM-CSF in BALF	Yokota et al., 2008	
	Female C57BL/6 mice (6-8 weeks)	Intratracheal administration 100µg DEPs on day 1, 4 and 7 weeks)	↑ BALF IL-1β upon DEPs compared to saline Role of IL-1 signalling in DEP-induced inflammation Independent of NLRP3 inflammasome and caspase-1	Provoost et al., 2011	
	IRC mice (6 weeks)	Intratracheal administration 2.5-10 mg/kg PM _{2.5} at day 0	Dose-dependent ↑ BALF IL-1β after PM instillation	Park et al., 2011	
	Female C57BL/6 mice (6-8 weeks)	Intratracheal administration 200 µg PM ₁₀ on day 0	PM-induced ↑ BALF IL-1β Role for NLRP3 inflammasome	Hirota et al., 2012	

Male wister rats (8 weeks)	Intratracheal administration 7.5 mg/kg body weight PM ₁₀ or PM _{2.5} for 14 days	↑ lung IL-1β in the PM groups compared to the controls	Tian et al., 2012
Male BALB/c mice (7-8 weeks)	Intratracheal administration 100μg PM ₁₀ on day 0, 3 and 6	↑ IL-1β in the BALF of PM ₁₀ -treated mice compared to sham ≈ IL-1β mRNA lung and blood	Farina et al., 2013
Female C57BL/6 mice (6-8 weeks)	Intranasal administration 200 μg PM ₁₀ on day 0	PM-induced ↑ IL-1β and GM-CSF in BALF No detection of IL-25, IL-33 and TSLP Role for NLRP3 inflammasome	Hirota et al., 2015
Male Wistar rats	Intratracheal administration 0.375-24 mg/kg body weight PM _{2.5} on day 1, 3, 5, 7 and 9	PM-induced ↑ IL-1β mRNA and protein level compared to saline exposure	Li et al., 2015
Male BALB/c mice (8 weeks)	Intratracheal administration 5.5 – 100μg PM _{2.5}	↑ IL-1β expression after PM exposure Positive correlation with tissue inflammation and BALF neutrophils	Van Winkle et al., 2015
Male and female BALB/c mice (6-8 weeks)	Intratracheal administration 4 mg/kg PM _{2.5} for 5 consecutive days	↑ IL-1β mRNA after PM exposure, not on protein level ↑ EGF-EGFR-Akt-NFκB signalling	Jin et al., 2016
Male BALB/c (6-8 weeks)	Intratracheal administration 0.5 mg PM _{2.5} at day 0 and 2	PM-induced ↑ IL-1β expression at day 7 and release in the BALF at day 3	Wang et al., 2017
Male Sprague-Dawley rats	Intratracheal administration 40mg/kg PM _{2.5} every other day for 3 tiimes	↑ IL-1β levels in BALF after PM exposure	Liu et al., 2017

Aggravating effects of DEPs/PM	Male ICR mice (6-7 weeks)	Intratracheal administration 100µg DEP every week for 6 weeks - 1µg OVA every 3 weeks for 6 weeks	OVA + DEPs : ↑ GM-CSF protein levels lung compared to vehicle; ≈ GM-CSF levels compared to OVA	Takano et al., 1997
	Male ICR mice (6 weeks)	12h/day, 7 days/week 0.3-3 mg soot/m ³ DE for 40 weeks 10µg OVA IP at week 16 1% OVA aerosol at a 3 week intervals during last 24 weeks	OVA + DE: dose-dependent ↑ GM-CSF protein levels lung compared to vehicle and OVA group	Takano et al., 1998
	Male ICR mice (6 weeks)	1 mg OVA IP at day 7 12 hours/day 2.2 m ³ DE inhalation for 5 weeks + 1% OVA aerosol at week 5	OVA + DE: ↑ GM-CSF protein levels lung compared to DE-saline group; ≈ GM-CSF levels compared to OVA	Miyabara et al., 1998
	Male C3H/HeN mice (6 weeks)		OVA + DE: ↓ GM-CSF secretion in lung supernatants compared to air – OVA	Miyabara et al., 1998 ⁰³
	Male C3H/He and BALB/c mice (8-10 weeks)	Intratracheal administration 1mg OVA IP at day 7 1x/week DEPs for 5 weeks + 1% OVA aerosol at week 5	OVA + DEPs : ↑ GM-CSF protein levels lung compared to vehicle-saline; ≈ GM-CSF levels compared to OVA	Miyabara et al., 1998 ¹⁰⁴
	Male BALB/c mice (7 weeks)	Intraperitoneal injection 10 µg OVA + 2mg DEPs on day 0.	OVA + DEPs: ↑ GM-CSF and IL-1α in the peritoneal exudate fluid compared to OVA Role for CD8+ T cells in GM-CSF production	Fujimaki et al., 2001
	C57BL/6N and CBA/JN mice (6 weeks)	Intratracheal administration 0-50µg DEPs + 1µg HDM (Derf) four times at 2 week interval	HDM + DEPs: ↑ GM-CSF in lung homogenate compared to HDM Not detected in CBA/JN mice	Sadakane et al., 2002

	Female BALB/c mice (6-8 weeks)	200-2000 $\mu\text{g}/\text{m}^3$ DEPs + 1% OVA aerosol for 10 days	$\approx$ GM-CSF BALF levels compared to OVA and saline	Whitekus et al., 2002
	Female BALB/c mice (6-7 weeks)	20 μg OVA IP on day 1 2 mg/m^3 DEPs + 1% OVA inhalation on day 14-17	$\approx$ GM-CSF BALF levels compared to OVA	Hao et al., 2003
	BALB/c, ICR and C3H/He mice (6 weeks)	Intratracheal administration 0-50 μg DEPs + 1 μg HDM (Derf) four times at 2 week interval	GM-CSF levels not detected in lung homogenate	Ichinose et al., 2004
	Female BALB/c mice (7-8 weeks)	IP 50 μg OVA on day 0 and 14 3 mg/m^3 OVA aerosol for 30min/day, 3 days/week for 4 weeks 50 μg PM_{10} or 30 mg/m^3 OVA aerosol on day 49	OVA + DEPs: $\uparrow$ IL-33 mRNA and protein BALF levels compared to carbon black; $\approx$ GM-CSF levels compared to chronic OVA exposure Role for IL-33 in inflammatory cell recruitment	Shadie et al., 2014
	Female C57BL/6 mice (6-8 weeks)	Intranasal administration 25 μg DEPs and 1 μg HDM on day 1, 8 and 15	DEPs + HDM: $\uparrow$ IL-25 and IL-33 protein lung levels compared to saline, sole DEP or HDM	De Grove et al., 2017

$\uparrow$: cytokine increases in response to DEPs/PM, $\downarrow$: cytokine decreases in response to DEPs/PM, $\approx$: equal cytokine levels