Cellular Mechanisms of the Adverse Respiratory Health Effects Induced By Ambient Particulate Matter: A Review and Perspective

Xu-Chen Xu, Zhou-Yang Li, Hai-Pin Chen, Jie-Sen Zhou, Yong Wang, Hua-Hao Shen and Zhi-Hua Chen*

Department of Respiratory and Critical Care Medicine, Second Hospital of Zhejiang University School of Medicine, China

Abstract

Environmental particulate matter (PM) pollution causes adverse respiratory health effects. Accumulating evidence suggests that exposure to high levels of PM, either acutely or chronically, is associated with increased loss of lung function, exacerbation, hospitalization, disease incidence, and/or mortality of certain chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease, and lung cancer. However, the detailed cellular mechanisms how PM induces the airway injury remain largely unknown. In this short review, we summarize recent advances on the fundamental mechanisms of PM-induced adverse respiratory health effects, with emphases on the damages in airway epithelial cells and the responses from the immune system. We further discuss the inadequacy of current studies and give a perspective on this burgeoning field.

Keywords: Particulate matter; Airway injury; Immune response; Mechanism

Introduction

Adverse respiratory health effects induced by ambient air pollution, specifically environmental particulate matter (PM), have drawn considerable attention over recent years. Epidemiological studies strongly suggests that high PM exposure is associated with decreased lung function and increased exacerbation, hospitalization, disease incidence, and/or mortality of certain chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and lung cancer [1-5]. According to the report of the Global Burden of Disease worldwide, particulate air pollution is estimated to cause 3.1 million deaths a year [6], and the World Health Organization calculates that 16% of the lung cancer deaths, 11% of COPD deaths, and about 13% of respiratory infection deaths are due to particulate air pollution exposure [7]. Despite of the many epidemiological or clinical studies, increasing researches have also focused on the airway injury mechanisms induced by PM. However, summarization of the mechanistic studies is little.

Here we perform a review on recent advances on the fundamental mechanisms of PM-induced adverse respiratory health effects. Articles eligible for this purpose are manually divided into several groups according to the target cells, with emphases on the airway epithelial cells and the immune response system. We further discuss the inadequacy of current studies and give a perspective on this burgeoning field.

Particulate Matter

Particulate matter, also referred as particle pollution or PM, is a complicated mixture of a number of components, including acids, organic chemicals, metals, and soil or dust particles [8]. PM can be described by its "aerodynamic equivalent diameter" (AED), and particles of the same AED are likely to have the same settling velocity. Traditionally, researchers tend to subdivide particles in AED fractions based on where they deposit in human airways: <10, <2.5, and <0.1 μm (PM_{10}/coarse, $PM_{2.5}$/fine, and $PM_{0.1}$/ultrafine, respectively) [7]. The size of particles is directly linked to their potential for causing health problems [9]. So here in our review, we go through original studies using particles that are 10 micrometers in diameter or smaller (PM_{10}) which means those particles can generally pass through the throat and nose and enter the lungs. It is important to notice that PM_{10} generally contains ultrafine ($PM_{0.1}$), fine ($PM_{0.1-2.5}$), and coarse ($PM_{2.5-10}$) fractions when interpreting PM researches [7].

For the reason of the many characteristics, various terms are employed reflecting the preparation or the source of PM samples, like PM$_{2.5}$, combustion-derived particulate matter (CDPM), diesel exhaust particle (DEP), MCP-230, and NIST-1649b, etc. In order to avoid unnecessary confusion caused by different terms used in the cited paper, we reconcile them into PM for simplicity in this review.

Particulate Matter on Lung/Bronchial Epithelial Cells

Airway epithelial cells are the first barrier to communicate with inhaled PM, and a large number of studies demonstrate that PM exerts significant effects on epithelial cells. Researches on this topic have generally focused on the human/murine/rat epithelial cell lines and primary epithelial cells. The existing of multiply choices of lung/bronchial epithelial cell lines and practiced culture skills of primary bronchial epithelial cells [10] facilitate to conduct researches on the effects of PM exposure.

Using human lung epithelial cells (L-132), Dagher et al. [11,12] performed a series of *in vitro* experiments and demonstrated a link between short-term PM exposure and inflammations through oxidative stress. They first showed that exposure to PM induced significant increases in gene expression and protein secretion of inflammatory cytokines. They hypothesized that the occurrence of the acute inflammatory response might rely on the capacity of such air pollutants to generate oxidative species, which have been implicated in the stringent regulation of the cytokine network [11]. They further demonstrated that PM were involved in the activation of the NF-κB/IκB complex, notably through the occurrence of oxidative stress conditions in L-132 cells [12]. Similar oxidative inflammatory responses could be detected in MLE (mouse lung epithelial) cells [13] and in primary bronchial epithelial cells [14].

Accumulating studies have investigated the role of PM in inducing epithelial cell apoptosis. For example, *in vitro* short-term exposure to PM induced apoptosis by activating not only the tumor necrosis factor-alpha (TNF-α)-induced pathway, but also the mitochondrial pathway [15]. Moreover, changes in the transcription rates of p53, Bcl-2, and Bax genes, and DNA fragmentation were reported in PM-exposed proliferating L-132 cells, revealing the occurrence of apoptotic events [15]. More recently, the same cell death pathways have been reported in human lung epithelial A549 cells upon PM treatment [16]. It is worth noticing that both cytotoxic effects and apoptosis were verified by Pálková et al. [17], as they tried to compare non-genotoxic and genotoxic effects of crude and fractionated extract of a standard reference DEP material - SRM 1650b on rat alveolar type II cell line RLE-6TN.

When apoptosis is mentioned, one cannot ignore the paradoxical autophagy since the connection between autophagy and apoptosis or other forms of cell deaths is a burgeoning area of research [18]. Deng et al. [16] discovered that PM exposure would induce autophagy as evidenced by an increased number of double-membrane vesicles, accompanied by increases of conversion and punctuation of microtubule-associated proteins light chain 3 (LC3) and expression of Beclin 1. Liu et al. [19] further elucidated the PI3K/Akt/m TOR signaling pathway could be a key contributor to PM-induced autophagy in human bronchial epithelial BEAS-2B cells. Considering theses studies, our group have found that autophagy was required for PM-induced expression of inflammatory cytokines and mucus hyper secretion via activation of NFκB1 and AP-1 pathways [20]. These

novel achievements provide a new insight into potential clinical applications by targeting autophagy inhibition for the prevention and/or treatment of PM-related lung disorders in the future. DNA damage is another widely investigated field related to adverse respiratory health effects. Borgie et al. [21] observed that transition metals and organic compounds in the collected PM sample induced cumulative DNA damage, by increasing the phosphorylation of H$_2$AX, the telomerase activity, and the miR-21 expression in BEAS-2B cells. Pálková et al. [17] also found that DEP contributed significantly to overall formation of DNA adducts associated with phosphorylation of p53, Chk1 or Chk2, and partly with apoptosis.

Other disease-related molecular mechanisms induced by PM in lung epithelial cells, such as senescence, contractility, transformation, and carcinogenesis, have also been investigated. For example, Rivas-Santiago et al. [22] showed that induction of senescence and down-regulation of human β-defensin 2 (HBD-2) and HBD-3 expression in PM-exposed A549 cells eventually led to enhanced M. tuberculosis growth, representing mechanisms by which exposure to air pollution PM may increase the risk of *M. tuberculosis* infection and the development of tuberculosis. Dysart et al. [23] figured that exposure to PM resulted in increased activation of transforming growth factor - beta (TGF-β), increased cell contractility, and elongation of lung epithelial cells. It also enhanced the existing interactions between extracellular matrix (ECM) stiffness and TGF-β, and thus augmented the susceptibility of developing pulmonary fibrosis. Morales-Bárcenas et al. [24] reported exposure to PM induced an increase in protease activity and invasiveness by up-regulating the activity of metalloproteases MMP-2 and MMP-9 and disrupted E-cadherin/β-catenin expression during neoplastic transformation. Also, accelerated cell proliferation, enhanced cell migration, and increased tumor growth were found in human lung carcinoma H460 cells by Luanpitpong et al. [25] after chronic exposure (3 months) to PM. The above studies provide new insights for the potentials of PM related to the pathogenesis of lung aging, fibrosis, or neoplasia.

Particulate Matter and Macrophages

Alveolar resident macrophages should react quickly to the inhaled PM as an initial innate immune response. While much of the enthusiasm in PM have been drawn to the epithelial cells, due attention have also been paid to macrophages in many studies. Not surprisingly, macrophages and epithelial cells share many features and pathophysiological mechanisms when exposed to PM.

For example, cytotoxicity and oxidative stress responses were also occurred in pulmonary macrophages after PM instillation in animal models [26]. Fritsch-Decker et al. [27] demonstrated that oxidative inflammatory response, similar in epithelial cells, was implicated in murine RAW 264.7 macrophages and in primary human macrophages, and they also believed that generation of ROS by PM is one of the primary mechanism initiating inflammatory processes. They further concluded that such reaction might be triggered via the MAPK cascade and the activation of arachidonic acid signaling pathway.

Cell apoptosis was also examined in PM-exposed rat alveolar NR8383 cells in a recent study [28]. Same mechanisms in epithelial cells including ROS generation and changes in transcription rates of Bcl-2 and Bax which suggest mitochondria-mediated apoptotic pathway could be well applied in alveolar macrophages. Also, it has been demonstrated that the degree of apoptosis was further aggravated

by inflammation, linking these two reactions into one integral whole [28]. The effects of PM exposure to macrophages also resembled epithelial cells in the aspect of latent TGF-β releasing accompanied by M2 polarization of AMs [29] and exertion of genotoxicity [30]. PM also induced mitochondrial fusion/fission dysfunction and mitochondrial lipid peroxidation in rat lung macrophages, which might be important mechanisms contributing to PM-induced respiratory diseases [31].

Particulate Matter on Dendritic Cells and Helper T cell Immunity

As one of the most powerful antigen presenting cells (APC), dendritic cells (DCs) are identified as key mediators between innate and adaptive immunities, which enabled DCs to be extensively involved in the pathogenesis of adverse respiratory health effects.

In 2008, Colin de Haar et al. [32] firstly found that PM could directly induce maturation of dendritic cells. Furthermore, Wang et al. [33] recently identified that PM induced the maturation of bone marrow derived dendritic cells (BMDCs), as evidenced by the expression of costimulatory molecules CD80 and CD86, in an uptake-dependent and oxidative stress-dependent manner, where the intracellular glutathione (GSH) and the GSH/oxidized GSH ratio were decreased. Increased antigen-specific T-cell proliferation was detected when the T cells were incubated with BMDCs exposed to PM *in vitro*. In a model of asthma induced by ovalbumin (OVA), PM exacerbated the pulmonary inflammation, which was attributed to the increases of neutrophils and macrophages but not eosinophils. These results correlated with the increased Th17 cells and related cytokines such as IL-17, and interestingly, compared with OVA-challenged mice, the levels of Th2 cells were inappreciably affected in PM treated asthmatic mice [33]. Enhanced DC activation, pulmonary inflammation and Th2-immune responsiveness as a result of innate immune activation by PM were also confirmed by Bezemer et al. [34], and the ability to alter lung tissue barrier integrity was suggested indeed.

A number of reports have found that DCs seem to have almost every connection with helper T cells when regarding the pathogenesis of asthma, thus any conclusion reached without adverting the interaction of both cells might be biased [35,36]. So following the above findings, it was found that maternal exposure to PM enhanced postnatal asthma development in mice, which might be related to the inhibition of pulmonary Th1 maturation and systemic oxidative stress in the dams [37]. Furthermore, data showed that the inhalation of PM by pregnant women caused an increase in the number of macrophages in BALF of offspring and contributed to the increased prevalence of childhood allergies by activating the Th2-associated immune response through increase in cytokines and chemokines (such as IL-5, IL-13, MCP-1, and RANTES) [38].

Similarly, early-life exposure to PM could also cause pulmonary immunosuppression [39]. Saravia et al. [39] found that early-life PM exposure induced an immunosuppressive environment in the lung, concurrent with increases in tolerogenic DCs and Tregs, which resulted in suppression of Th2 responses. However, despite having early immunosuppression, these mice develop severe allergic inflammation when challenged with allergen as adults [39]. These findings demonstrate a mechanism whereby PM exposure modulates adaptive immunity, and leads to a predisposition to develop asthma upon rechallenge later in life.

Inoue et al. [40] have found that PM exposure facilitates APC activity including DC and extra thoracic antigen-specific Th response which shed light on the mechanisms regarding the adverse effects of PM on allergic asthma by evaluating the effects of PM on the phenotype and function of BMDC *in vitro* and on the expression pattern of APC-related molecules in the murine lung in the presence or absence of antigen *in vivo* [40]. Vital genes like nuclear factor elytroid 2 (Nrf2) deficiency in DC may also promote a constitutive immune-polarizing cytokine milieu, leading to the augmented adjuvant effect of PM on allergic sensitization [41]. Taken all together, it is obvious that PM may constitute an important and unrecognized risk factor in the exacerbation and development of asthmatic phenotypes.

Particulate Matter on Other Immunocytes

Apart from those conventional mechanisms discussed above, PM was proved to have distinctive way to affect other objects especially within the immune system. Other than alveolar macrophages referred in the previous section, NK cells is also involved in the PM-mediated susceptibility to *S. aureus* bacterial infection [42]. By intratracheally instilling with a single dose of PM and followed by an intratracheal inoculation with bacteria *S. aureus*, Zhao et al. [42] found that PM exposure significantly decreased the natural killer (NK) cells recruited into the airways, and AMs from PM-experienced lungs had depressed phagocytosis of *S. aureus* [42]. Furthermore, the presence of NK cells markedly enhanced the ability of AMs to phagocytose *S. aureus ex vivo*. Thus, this study identifies an important role of NK cells in the lung upon PM exposure, providing a potential mechanism to explain the epidemiological findings that associate PM pollution and increased lung bacterial infections.

Conclusion Remarks

As summarized above, the studies of PM pathogenesis mainly focus on the epithelial cells and inflammatory responses in PM-related diseases, such as asthma and chronic obstructive pulmonary disease. These studies provide a wide range of information on the pathophysiology of PM-induced respiratory disease and, as a consequence, support the development of new protective and therapeutic approaches for PM-related disorders.

However, there are still some divergent aspects regarding the mechanistic research in PM. 1) Sources of the PM seem to be of great variety. There is no such defined thing as "Standard Particulate Matter", and researchers are more likely to utilize collected urban particles when conducting the experiments. Since the elements constituting the PM do make a significant difference in triggering multifarious mechanisms, an explicit criterion of PM may be of urgent need. 2) Most methods of PM inhalation are tracheal instillation, which can only damage the airway but are unable to mimic the process of human aspiration of PM naturally. 3) At present, the exposure time of PM in these studies is relatively short, which is not consistent with the rule that only exposure to PM for a long term can lead to chronic airway inflammation. 4) Despite the current understandings on the mechanisms of PM-induced pulmonary injury, the key signaling pathways, which regulate PM-induced cell apoptosis, inflammatory, DNA damage, and carcinogenic effects still remain largely unknown. 5) The fate of inhaled PM is not clear, especially *in vivo*. Is the inhaled PM endocytosed by epithelial cells or by macrophages? What sized PM can be endocytosed by epithelial cells and macrophages? If endocytosed, does the PM get digested or secreted eventually? How does PM induce cellular damages when it is in the cell? How is PM

finally removed from the respiratory tract? 6) Translational researches are urgently needed to transfer the current mechanistic advances into clinical trials, hopefully leading to therapeutic approaches for PM-related disorders. 7) Also, since the respiratory system is tightly connected with the cardiovascular system in terms of PM-induced adverse health effects, future researches could lay a momentous emphasis on the reciprocities of these two pivotal systems.

Nonetheless, accumulating mechanistic studies shed new lights on our understanding of the PM-induced pathogenesis, and will eventually benefit the people who are suffering from environmental particulate pollution.

Acknowledgement

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