Impact of Particulate Matter 2.5 on A549 Human Lung Epithelial Cells and Alveolar Macrophage like Cells

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Introduction

Air pollution is considered as a global health problem worldwide due to its rapid increase. Particulate matter (PM) represents one of the main components of this air pollution[1]. Therefore, the impact of PM on the functionality of different systems in the human body is constantly being investigated. Although PM can be distinguished by their chemical composition and origin, PM are mostly divided into three groups according to their size: PM_{10} with a diameter ≤ 10 µm, $PM_{2.5}$ with diameter ≤ 2.5 µm and ultrafine particles (UFP, 1-100 nm)[2] **(Figure 1)**. $PM_{2.5}$ can float in the air for a long time and by breathing in, they can reach the alveoli and impair the function of different cell types in the respiratory tract including alveolar epithelial cells type II (AET II) and alveolar macrophages (AM cells)[3].

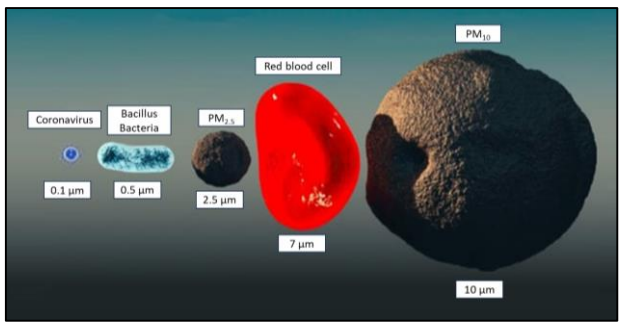


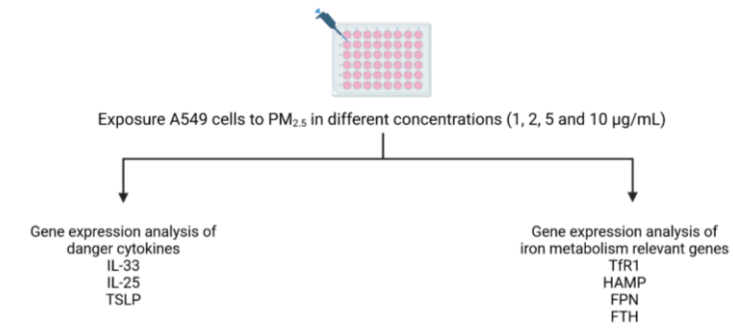
Figure 1. Size of $PM_{2.5}$ in comparison with PM_{10}[4]

This work aims to further investigate the effects and mechanisms behind the harmful effects of $PM_{2.5}$ on the respiratory system. This includes the effect on iron homeostasis and on the release of alarm cytokines IL-25, IL-33 and thymic stromal lymphopoietin TSLP from AET II, as well as the influence on the phenotype of Alveolar Macrophage like Cells (AML cells).

Methods

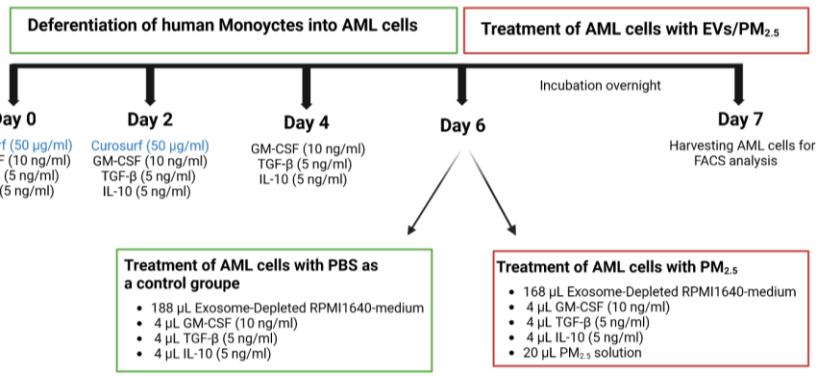
Exposure AET II cells to $PM_{2.5}$

AET II cells (A549 cell line) were incubated with $PM_{2.5}$ at different concentrations (1, 2, 5 and 10 µg/mL) for 12 hours. A qPCR assay was used to analyze the effect of $PM_{2.5}$ exposure on the gene expression of the alarmins IL-33, IL-25, TSLP and on the gene expression of iron metabolism relevant genes Transferrin receptor 1 (TfR1), Ferroportin (FPN), Ferritin heavy chain (FTH) and Hepcidin antimicrobial peptide (HAMP)



In vitro Differentiation of human Monocytes into AML cells and Exposure to $PM_{2.5}$

Monocytes were sorted from human blood samples and cultured with the differentiation cytokines for six days[5]. AML cells were exposed overnight to $PM_{2.5}$ (10 µg/ml). AML cells were analyzed with Fluorescence Activated Cell Sorting (FACS). LEGENDplex Human Macrophage/Microglia Panel (13-plex) was used to analyze the secreted proteins/cytokines in the supernatant.



Results

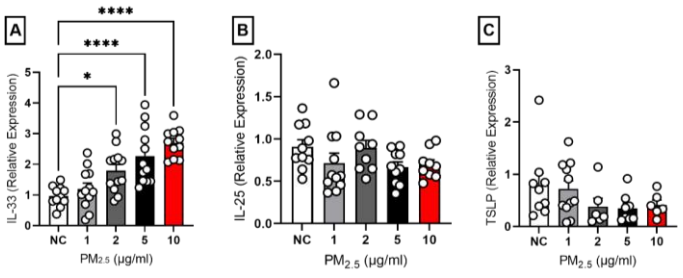


Figure 2. qPCR analysis of IL-33 (A), IL-25 (B) and TSLP (C) gene expression in A549 cells after $PM_{2.5}$-Exposure.

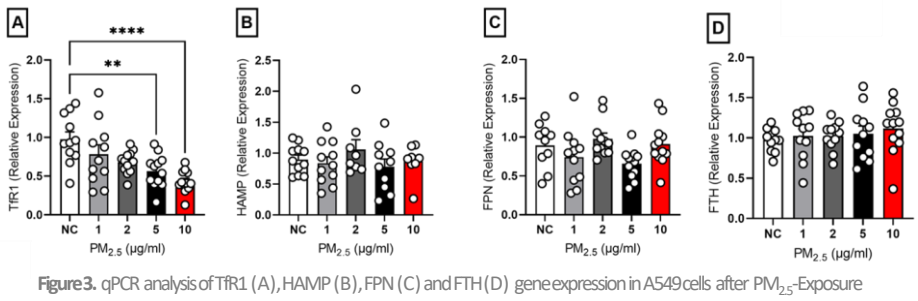


Figure 3. qPCR analysis of TfR1 (A), HAMP (B), FPN (C) and FTH (D) gene expression in A549 cells after $PM_{2.5}$-Exposure

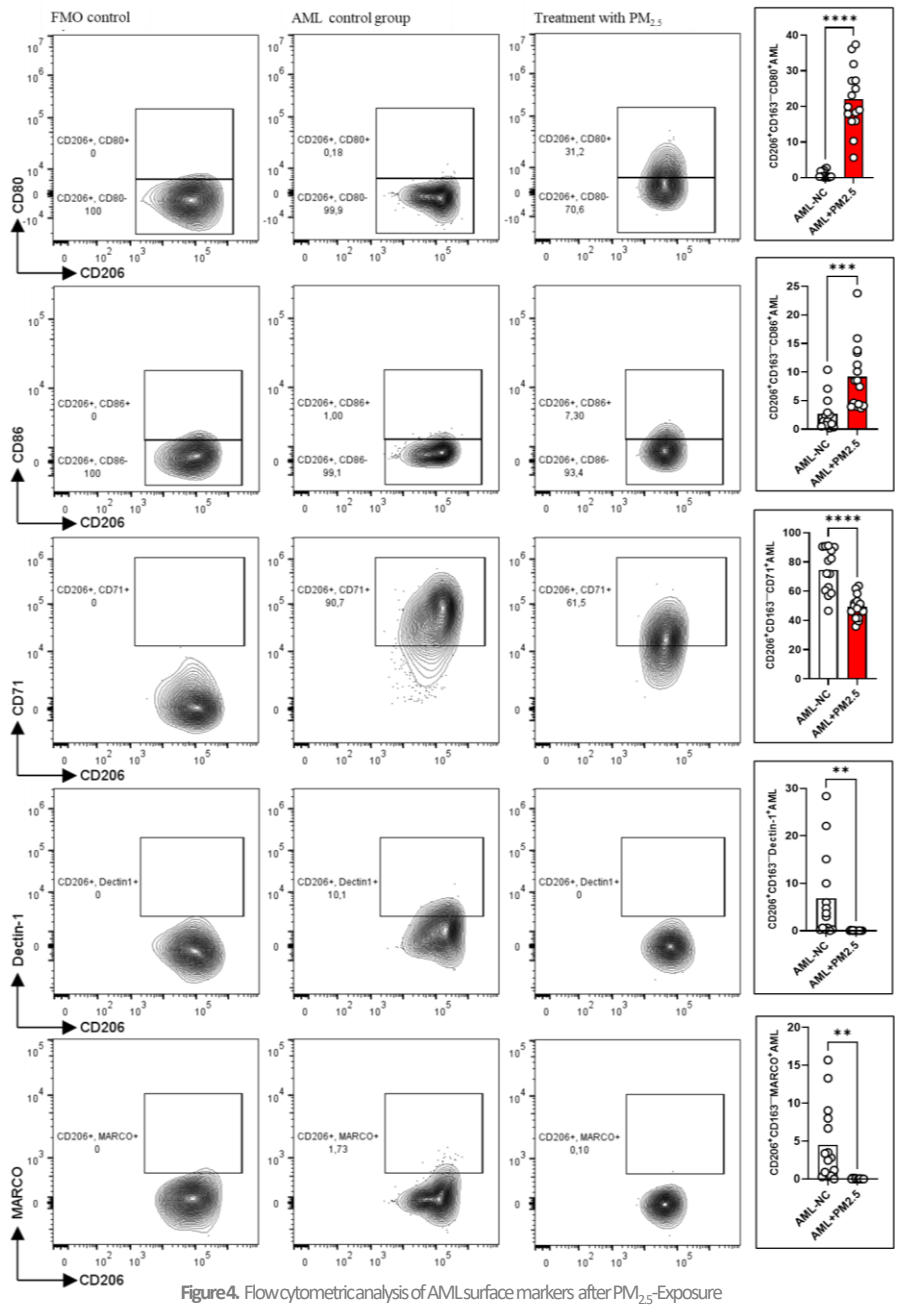


Figure 4. Flow cytometric analysis of AML surface markers after $PM_{2.5}$-Exposure

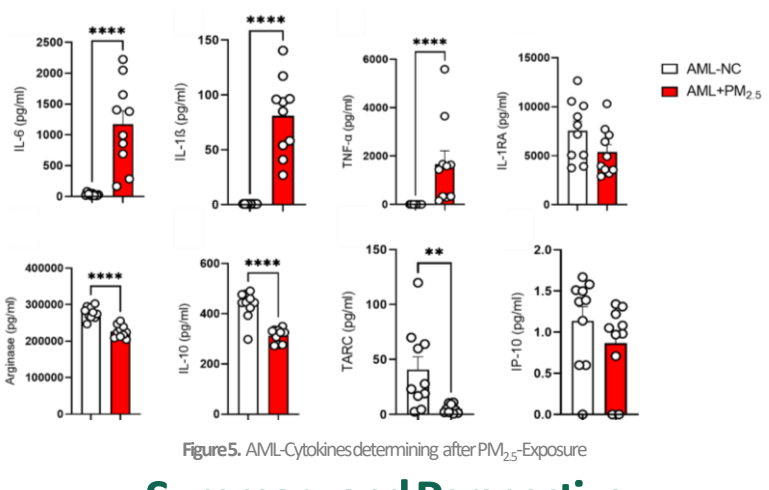


Figure 5. AML-Cytokines determining after $PM_{2.5}$-Exposure

Summary and Perspective

The results of this work demonstrate an overview of the harmful effects of $PM_{2.5}$ on the human lungs (Figure 6) and provide a starting point for further investigations into $PM_{2.5}$ and their role in the development or exacerbation of various respiratory diseases.

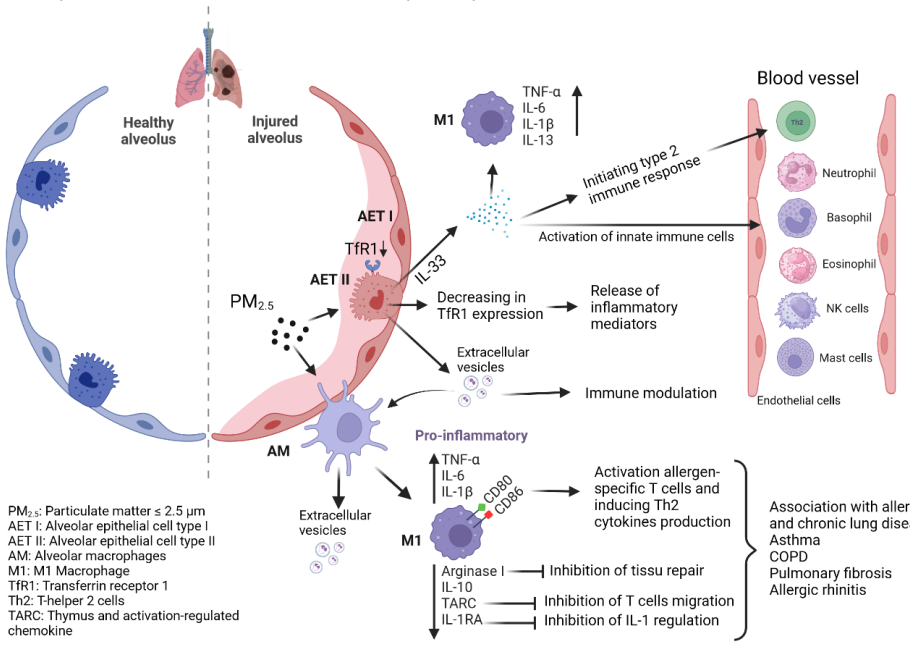


Figure 6. The influence mechanisms of $PM_{2.5}$ on both AET II and AM/AML cell and their association with allergic and chronic lung diseases. The pathways of action of $PM_{2.5}$ presented were based on the results obtained in this work and the findings reported in other studies.

[1] Cho, C.-C. et al. In Vitro and In Vivo Experimental Studies of PM2.5 on Disease Progression. IJERPH 15, 1380; 10.3390/ijerph15071380 (2018). [2] Madureira, J., Slezakova, K., Costa, C., Pereira, M. C. & Teixeira, J. P. Assessment of indoor air exposure among newborns and their mothers: Levels and sources of PM10, PM2.5 and ultrafine particles at 65 home environments. Environmental Pollution 264, 114746; 10.1016/j.envpol.2020.114746 (2020).[3] Li, T., Yu, Y., Sun, Z. & Duan, J. A comprehensive understanding of ambient particulate matter and its components on the adverse health effects based from epidemiological and laboratory evidence. Part Fibre Toxicol 19, 67; 10.1186/s12989-022-00507-5 (2022). [4] OXYCOM. OXYCOM natural air conditioning. Available at https://www.oxy-com.com/blog-news/intrcooll-against-contamination-via-aerosols. [5] Pahari, S. et al. A new tractable method for generating Human Alveolar Macrophage Like cells in vitro to study lung inflammatory processes and diseases. bioRxiv : the preprint server for biology; 10.1101/2023.04.05.535806 (2023).