A MULTIPARAMETRIC ANALYSIS OF HUMAN MONOCYTE-DERIVED MACROPHAGE RESPONSE TO CARBON BLACK PARTICLES IN VITRO

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Background. Macrophages as cells of the innate immune system and a first-line defense against various invading pathogens and other substances are responsible for phagocytosis. Phagocytosis is a process of particle elimination via ingestion and intracellular degradation and is widely studied in vitro. Most of the current quantitative phagocytosis assays consist of particles that are manufactured to have the same shape and size and are based on fluorescence or other types of dyes or biomarkers. These types of assays are useful but are lacking in variability, especially for the studies of phagocytosis of diverse environmental particles. One type of these particles is black carbon. Black carbon is a core component of air-polluting particulate matter and is associated with adverse health effects and increased susceptibility to respiratory infections, development of chronic obstructive pulmonary disease, and asthma. Lung-resident macrophages play a crucial role in the clearance and response to various inhaled particles, including black carbon. Phagocytosis by macrophages represents a fundamental process that determines the further fate of inhaled particles and the impact that they might have on human health.

Objectives. We aimed to investigate the interaction of BC particles and human monocyte-derived macrophages in vitro. The findings from this study open new insights into the role of macrophages in black carbon-exposed airways and lungs and have implications for the pathogenesis of many diseases.

Methods. We compared two types of commercially purchased black carbon particles by several physicochemical methods and by their biological effects on monocyte-derived macrophages. Confocal microscopy and CellProfiler, an open-source cell imaging tool, were employed for quantitative analysis of phagocytosis. Black carbon-induced changes in cell viability, morphology, and particle uptake/phagocytosis were quantified. Inflammation and oxidative stress biomarkers were assessed in parallel by Western blot (Nrf2, NQO1, HO-1, p62, p-p62, LC3A/B) and ELISA (IL-6, IL-8, IL-1B).

Results. We report significant and comparable monocyte-derived macrophage responses to both types of black carbon applied in terms of particle uptake, inflammatory cytokine production, and oxidative stress response-related protein expression. Our results show that black carbon particles induce innate immunity activation and oxidative stress in macrophages, potentially leading to the development of chronic inflammatory lung diseases.

Keywords. Monocyte / Macrophage; Phagocytosis; Particle analysis; Carbon black; CellProfiler;