Carbon-black (CB) is the primary nanoparticulate component of air pollution from fossil fuel combustion. Prior research has demonstrated that CB induces increased macrophage oxygen species and cellular stress upon cellular accumulation via receptor-mediated endocytosis. Recent published work has identified particulate tracers of asbestos and some rare earth elements to have the capacity to activate pro-inflammatory/inflammatory signaling from immune system cells. This work quantifies the cellular impact of ultrafine carbon (carbon-black, CB) nanoparticles, that range in size down to 30 nm, on macrophage cell viability. CB nanoparticles were prepared via sonication in a buffered triton X100 solution. The size analysis of the carbon black nanoparticles was performed using atomic force microscopy (AFM) and transmission electron microscopy (TEM) techniques. RAW246.7 macrophage cells were exposed to CB doses ranging from 50–490 µg/ml in complete media. Analysis of cell survival over time revealed altered states of significant nuclear degradation and cell fitting after 40 hours of exposure, and in a dose dependent pattern. Live imaging of cells exposed to nanoparticles revealed a viable phenotype of accumulation over time. To assess inflammatory signaling, both caspase-1 activation and IL-1β production was observed in whole cell lysates. Caspase-1 activation was measured as the appearance of the active (cleaved) form of the protein appearing in immunoblots. Caspase-1 activity is responsible for proteolytic processing of pro IL-1β to IL-1β, subsequent to release from macrophages. Immunohistochemistry revealed significant activation of caspase 1 with 48 hour CB exposures at doses of 100-200 µg/ml. Similarly, levels of IL-1β were significantly induced by CB exposure, with maximal induction observed after a 48 hour exposure. In particular, accumulation in macrophages has been shown to change the autophagic degradation machinery, macrophage cells were assayed for autophagy via induction of LC3, a marker for autophagosome vesicles. Immunofluorescence analysis revealed significant accumulation of LC3 in response to CB exposure and in response to chloroquine, which inhibits autophagosome lysosomal fusion. Further analysis of autophagic pathways via microscopy will be discussed. Taken together, these results suggest a model in which CB exposure stimulates the inflammatory and disrupts autophagy in macrophages.

Introduction

Lysosomes are organelles that act as the terminal location in cytoplasmic pathways and are thus involved in cellular recycling and defense. Lysosomes complete these tasks through proteinase to maintain (4-5X) pH, specialized enzymes that degrade proteins, and lysosomal hydrolases with astrophilic properties with astrophilic properties. When lysosomal membranes are damaged, pathogens and toxins leak into the cell, and since lysosomal metabolic pathways are required for the normal functions of the cell, the damage can result in cell death. Lysosomal-mediated autophagy is a starvation and damage induced mechanism that allows the cell to degrade its components and mount an immune response. The main characteristic features of autophagy (phagy) is the attachment of a membrane to the target and the cytoplasmic protein LC3 II to LC3 I. After the closing of the autophagic vacuole, lysosomes fuse and the contained items are degraded by lysosomal hydrolases.

Model 1: Overview of Autophagy

Carbon-black (CB) is the primary nanoparticulate component of environmental pollutants and is emitted through carbon combustion reactions. CB has been shown to induce inflammatory, oxidative stress, aggregation of proteins, altered cell polarization, fragmented mitochondria, and increased mannose receptor (MAR) in human macrophages. In addition, cell death can lead to autophagic vacuoles (AVs) formation. AVs have been shown to prevent the critical process of cell death by autophagy. Finally, the degradation of autophagosomes is a necessary process for survival and has been linked to cancer, neurodegenerative diseases such as Parkinson’s disease, and lysosomal storage diseases (LSDs) such as Tay-Sachs disease. These findings indicate the damaging cellular role of AVs as they relate to lack of cellular control, cellular inflammation, and overall detrimental effects.

Model 2: Relationship between NPs, Inflammation, and Apoptosis

Carbon-black nanoparticles activate inflammasome signaling and cell death in macrophage cultures.