

## Air Pollution Particulate Matter Exposure Up-Regulates the Expression of CD206 and TNF- $\alpha$ Production in Monocytes during *in vitro* Differentiation into Macrophages

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**Introduction:** Alveolar macrophages play a central role in the protective immune response to respiratory infections and are the first line cellular responders to inhaled particulate matter (PM) and microbial pathogens. Monocytes are recruited from blood during inflammation and then mature into alveolar macrophages. Mechanisms by which PM modulate innate responses of macrophages are not understood.

**Objectives:** To examine the effects of PM exposure on human monocytes during *in vitro* differentiation into macrophages and assess phagocytosis of PM, expression of cell-surface molecules and TNF $\alpha$  production.

**Methods:** Peripheral blood was obtained from healthy adult volunteers (n=10). Monocytes were isolated from peripheral blood mononuclear cells by plastic adherence and positive immunomagnetic selection and exposed to PM during *in vitro* differentiation into macrophages for seven days. Cell morphology, proportions of cells containing PM and TNF- $\alpha$  production were assessed by microscopy, flow cytometry for cell-surface expression of CD14, CD16, CD33, CD36, CD163, CD206 and CD209 and ELISA, respectively. Ambient PM<sub>2.5</sub> (aerodynamic diameters <2.5 $\mu$ m) for *in vitro* exposure studies, were collected with high-volume PM<sub>2.5</sub> samplers (GMW Model 1200, VFC HVPM10, airflow rate 1.13m<sup>3</sup>/min) at the National Center for Environmental Research and Training (CENICA, Mexico City).

**Results:** Monocytes exposed to PM during their *in vitro* differentiation exhibited a round morphology, while unexposed monocytes showed a non-rounded morphology and were elongated. The proportion of cells containing PM increased according to the concentration of PM with the highest proportion of cells containing PM being 22% (15-32, 10mg/mL). Exposure to 1, 5 and 10mg /ml of PM did not show significant changes to the cell-surface expression of CD14, CD16, CD163 and CD33. Although exposure to 1, 5 and 10mg/ml of PM did not significantly alter the expression of CD36 a trend to a decrease in proportion of cells expressing CD36 was observed when PM concentrations were increased. The expression of CD206 was significantly increased in cells exposed to 10mg /mL of PM ( $p<0.005$ ). In addition, exposure to 10mg /mL of PM induced significant production of TNF- $\alpha$  in comparison to non-exposed cells ( $p<0.005$ ).

**Conclusions:** *In vitro* exposure to PM enhanced the expression of CD206 and production of TNF $\alpha$  during macrophage differentiation. Our data support the hypothesis that PM affects the differentiation of monocytes to macrophages and modify macrophage function.