Long-term effects of repeated exposure to fine and ultrafine particles on lung epithelial cells and fibroblasts.

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Keywords: atmospheric particles, airway remodeling, differentiation, inflammation
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Chronic exposure to particulate matter (PM) is suspected to exacerbate chronic inflammatory diseases such as asthma characterized by an airway remodeling. This remodeling affects the different tissues constituting the airway walls and especially bronchial cells that produce more mucus and fibroblasts that proliferate. However the underlying mechanisms are far to be understood. In this context, to better understand the long term effects of PM, we developed an experimental strategy using primary culture of human bronchial epithelial repeatedly exposed to PM to investigate whether particles could contribute to airway remodeling by inducing sustained inflammation and mucus secretion. Moreover we investigated whether the epithelial secretions induced by particle exposure could have a proliferative effect on lung fibroblasts

Particles were sampled in Paris with 13 stages Dekati impactors in order to collect coarse (10-1µm), fine (1-0.1µm) and ultrafine (<0.1µm) particles. Their effect was observed on primary cultures of normal human bronchial epithelial cells (NHBE) grown at an air-liquid interface in two compartments chambers (Transwell). After four 48 hrs-spaced treatments of 4 hrs from 1 to 10 µg/cm², the evolution of the pro-inflammatory response, the epithelium differentiation and the fate of particles were studied during the 5 following weeks. Moreover, we investigated whether the epithelial secretome could have a mitogenic effect on fibroblasts using two different approaches: cell counting of fibroblasts were performed either when grown in presence of conditioned media (basal media from untreated or PM-treated cells) or when co-cultured with epithelial cells treated or not with fine PM.

Ultrastructural observations revealed that particles were still present in the bronchial epithelium 5 weeks after treatments. Such particles likely represent the insoluble core of the particles as we supposed that organic compounds have been metabolized. Indeed, we observed an increased expression of cytochrome P450 1A1, a xenobiotic metabolizing enzyme involved in organic compounds metabolism, in fine and ultrafine PM-treated cultures. CYP1A1 was highly dose-dependently overexpressed during particle treatment and return to basal level two weeks after the end of the treatments. GM-CSF and IL-6, two biomarkers of a pro-inflammatory response, are slightly but significantly released after each treatment but maintained and increased (for IL-6) and up to 6 weeks. Amphiregulin (a growth factor of the EGF Receptor ligand family involved in the pro-inflammatory response and mucus release (Ramgolam *et al.*, 2012, Val *et al.*, 2012)) was released after each treatment but decreased after the end of the treatments. The number of cells expressing MUC5AC, an important mucin in bronchia, was increased in fine and ultrafine PM-treated cultures.

The proliferation of fibroblasts was increased when exposed to conditioned media from fine PM-treated cells recovered during the 4-treatment period and not by conditioned media from fine PM-treated cells recovered after the treatment period. This proliferation was inhibited when conditioned media were used in presence of an antibody directed against EGF receptor suggesting that EGFR ligands such as amphiregulin were responsible for this mitogenic effect. The proliferation of fibroblasts was also increased when they were co-cultured with epithelial cells treated with fine PM.

Our results demonstrated that bronchial epithelial cells repeatedly exposed to urban fine and ultrafine particles exhibited a sustained pro-inflammatory response, evolved towards a mucous phenotype and produced mediators involved in fibroblast proliferation. Altogether these data suggest that PM could favor the airway remodeling.

This work was supported by the National Agency for Research ANR under grant CESA 009 02, project “Megatox”.

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