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Inflammatory effect of sputum microparticles derived from pulmonary diseases in a murine model

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Microparticles (MPs) are small plasma membrane vesicles released by several cell types (macrophages, platelets, endothelial cells, granulocytes, monocytes, lymphocytes) following chemical, physical and apoptotic stimuli. MPs bear a number of bioactive effectors that can be disseminate, exchanged, and transferred via MPs cell interactions. The hallmarks of lung disease in cystic fibrosis (CF) patients are a persistent infection with opportunistic bacterial pathogens such as the Gram-negative P. aeruginosa and an abnormal inflammatory response dominated by polymorphonuclear granulocytes (neutrophils). In CF airways, neutrophils undergo conventional activation and functional reprogramming. In our previous study, we isolated MPs in sputum and demonstrated that most of CF sputum MPs were of granulocyte origin.

However, the neutrophil response is not capable to clear bacteria from the CF airways ensuing in exaggerated apoptosis.

The aim of this study is to investigate if MPs isolated from the sputum of patients with pulmonary diseas, in particular CF and dyskinesia primary ciliar (DPC), contribute to induction of lung inflammation.

Swiss CD11 mice (6–8 weeks old male) were induced by intratracheal administration of MPs isolated from the sputum of CF and DPC, LPS (20μg) or saline.

Histologycal analysis was performed on H and E stained lung sections.

In Bronchoalveolar lavage fluid (BALF) total cells were counted by trypan blue stain, whereas differential cell counts were performed on cytospin preparations stained with May-Grunwald Giemsa.

LPS induced a significant increase of total cellular count as compared with controls. MPs obtained from CF patients in acute state were inflammatory as well, with a peak of total cell counts obtained with 100 x 106 MPs injected. Interestingly, MPs obtained from CF intermittent state were less pro-inflammatory in comparition to acute CF patients MPs, while DPC patients MPs determined an intermediate inflammatory levels, between those elicited by acute and intermittent CF MPs.

The analysis of differential cell counts revealed that endotoxin, 50 and 100 x 106 MPs from Cfpatients, determined an increase in neutrophil numbers; no differences were found between acute and intemittent CF patients, whereas controls had only macrophages in their BALF. MPs from DPC patients have a different behaviour, since the most of inflammatory cells were macrophages.

Our data suggest that MPs isolated from CF sputum could contribute to lung injury resultant in increase of total inflammatory cells and neutrophil recruitment.

Keywords: Microparticles, inflammation, lung diseas.