IJAE Vol. 115, n. 1/2 (Supplement), 2010

A new biological marker isolated and characterizated in sputum from lung pathology patients: microparticles

Chiara Porro[1], Silvia Lepore[1], Teresa Trotta[1], Stefano Castellani[1], Luigi Ratclif[2], Sante Di Gioia[1], Maria Carmen Martínez[3], Massimo Conese[1], Angela Bruna Maffione[1]

[1] Department of Biomedical Sciences, University of Foggia, Foggia, Italy
[2] Centro Regionale di Supporto FC, Ospedale "G. Tatarella", Cerignola, Italy
[3] CNRS UMR 6214, INSERM U 771, Université d'Angers, Angers, France

Sputum is recognized as a very useful sampling method in Cystic Fibrosis (CF) for both research and clinical use aiding both the diagnosis and monitoring of lung disease inflammatory status. A great advantage of the technique is that it enables sampling of the airways in a non-invasive manner. We investigated on the presence and phenotype of microparticles (MPs) in the sputum, of acute and intermittent CF and Primary Ciliary Dyskinesia (PCD) adult patients. MPs are membrane vesicles that are released during cell activation and apoptosis. MPs have different biological effects depending on the cell from they originate. CF lung disease is characterized by massive neutrophil influx in the airways, their activation and eventually apoptosis. Spontaneous sputum, obtained from 21 CF patients (10 acute and 11 intermittent) and 7 patients with PCD, was liquefied with Sputasol. MPs were counted, visualized by electron microscopy, identified in the supernatants of treated sputum by cytofluorimetry and immunodecoration for leukocyte (CD11a), granulocyte (CD66b), and monocyte-macrophage (CD11b) antigens. Electron microscopy revealed that sputum MPs were in the 100-500 nm range and did not contain bacteria. CF sputa contained higher number of MPs in comparison with PCD sputa. CD66b and CD11a, but not CD11b, levels in MPs were significantly higher in CF than in PCD. Acute and intermittent patients presented significantly higher levels of CD11a- and CD66b-expressing MPs respect to PCD patients. MPs are detectable in sputa obtained from CF patients and are predominantly of granulocyte origin. These findings are consistent with massive influx of neutrophils into CF airways and their accumulation on the surface of the airway epithelium. In this environment, neutrophils are activated by bacterial products, pro-inflammatory cytokines, and chemokines. Neutrophils undergo apoptosis, as normally happens in acute inflammation, but also post-apoptotic necrosis, releasing toxic enzymes and oxygen radicals. MPs in CF sputa likely reflect both activation and apoptosis of neutrophils. The significantly less presence of granulocyte-derived MPs in PCD respect to CF patients, may be due to the fact that lung disease in PCD, although similar, is delayed in time as compared with CF.

In CF airways, neutrophil-derived MPs could contribute to self-perpetuating inflammatory cycle, and may account for the exaggerated pro-inflammatory response of cells in CF patients. The MPs isolated from sputum could be a new non invasive methods and opens a new opportunity for the study of lung disease.

Key words
Microparticles, sputum, cytofluorimetry, electron microscopy