ImmunoTools *special* Award 2013

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The role of Damage Associated Molecular Patterns (DAMPs) in the genetic susceptibility towards Chronic Obstructive Pulmonary Disease (COPD)

Chronic Obstructive Pulmonary Disease (COPD) is a debilitating and progressive lung disease, characterized by accelerated lung function decline and airflow limitation. The main cause of COPD is chronic exposure to noxious gasses and particles, including cigarette smoke (CS). During the early phases of disease, especially the innate immune system is activated as demonstrated by increased recruitment and activation of neutrophils, macrophages, natural killer cells and mature dendritic cells in lung tissue and airway lumen. The innate immune system can be triggered by Damage Associated Molecular Patterns (DAMPs) released from damaged or dead cells, activating pattern recognition receptors. Epithelial cells are the first line of defence in the lungs and are therefore the cells that are most damaged by CS. Lately, the role of DAMPs in COPD is emerging, as multiple DAMPs have been found to be increased in extracellular lung fluids. Nevertheless, the specific role of DAMPs in the pathophysiology of COPD has yet to be unravelled.

We hypothesize that bronchial epithelial cells isolated from COPD patients are more prone to DAMP-mediated responses than bronchial epithelial cells isolated from control smokers.

Bronchial epithelial cells isolated and cultured from COPD patients and control smokers using bronchial brushings will be stimulated with 0-50% cigarette smoke extract (CSE) for 4 hours. Thereafter, cell death will be determined using **FITC conjugated Annexin V** – PI staining using flow-cytometry, distinguishing between apoptotic and necrotic cells. An increased rate in necrotic cell death is expected to result in DAMP release, which can be determined in the supernatant using commercial ELISA kits for measuring DAMPs (HMGB1, HSP70, Galectin-1, IL-1α, IL-33). COPD patients show on-going chronic lung inflammation with increased expression of pro-inflammatory cytokines which may increase the damage initiated by CS. Therefore, bronchial epithelial cells will be co-stimulated with CSE and **recombinant human cytokines** (**IFN-γ**, **IL-1α**, **IL-1β**, **IL-2**, **IL-6**, **IL-8**, **IL-17A**, **CXCL10, MCP1**, **TGF-β** and **TNF-α**) and effects on cell death and DAMP release will be determined. Furthermore, bronchial epithelial cells from COPD patients and healthy volunteers will be stimulated with **recombinant human DAMPs** (**Galectin-1**, **IL-1α**, IL-33, HSP70, HMGB1) after which the pro-inflammatory activity will be

analysed using flow cytometry (**PE conjugated IL-6**) and ELISA (**human ELISA** for **IL-4**, **IL-6**, **IL-8** and **TNF-α**).

Additionally, the same experiments will be performed using mouse bronchial epithelial cells isolated from BALB/c mice (susceptible to develop neutrophilic airway inflammation upon CS exposure) and C57BL/6 mice (resistant to develop neutrophilic airway inflammation upon CS exposure). Stimulating bronchial epithelial cells with 0-50% CSE alone or in combination with **recombinant mouse cytokines** (**IL-1α**, **IL-1β**, **IL-2**, **IL-6**, KC, **IL-17A**, **MCP1**, **CXCL10**, **IL-33** and **TNF-α**), after which cell-death will be determined using **FITC conjugated Annexin-V** – PI staining, and the DAMP release profile (HMGB1, HSP70, Galectin-1, IL-1α, IL-33) will be measured in the supernatant using commercially available ELISA kits. Moreover, mouse bronchial epithelial cells will be stimulated with **recombinant mouse DAMPs** (Galectin-1, **IL-1α, IL-33**, HSP70, HMGB1) after which the immune reaction will be analysed using commercially available ELISA kits (mouse ELISA for IL-4, IL-6, KC and TNF-α). These experiments in mice are necessary for validating the results in a mouse model, as a mouse model will be used for follow-up studies.

This study will improve insight concerning the role of DAMPs in the pathophysiology of COPD, by measuring the release of DAMPs upon CS-exposure as well as the response to DAMPs between COPD patients and control smokers. The current study will possibly provide new research targets for the treatment of COPD.