ImmunoTools *special* Award 2015

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Crosstalk between human lung epithelial cells and fibroblasts; implication for COPD

COPD is a life threatening disease characterized by chronic airway inflammation, irreversible airflow limitation and accelerated lung function decline. Currently, there is no cure for COPD. Abnormal tissue repair upon cigarette smoking, a major risk factor for COPD, leads to thickening and obstruction of the small airways on one hand and alveolar destruction (emphysema) on the other hand.

Inhaled cigarette smoke first encounters the airway epithelium, which forms a physical barrier and is part of innate immunity. Additionally, damaged epithelium can secrete growth factors and cytokines that act on immune cells as well as underlying fibroblasts. Fibroblasts are key structural cells involved in tissue repair and remodeling by the production of extracellular matrix (ECM) proteins. They respond to smoke exposure, growth factors and cytokines by increasing inflammatory mediator production and changing the production of structural proteins, such as α-smooth muscle actin (SMA) and fibronectin. However, it is still unclear why cigarette smoking leads to chronic inflammation with tissue remodeling in COPD patients but not in healthy individuals.

We hypothesize that the structural changes and the chronic inflammation in COPD involve an aberrant communication between the airway epithelium and underlying fibroblasts. So far, most research have focused on the role of individual cells in COPD, but this project will examine their interaction.

Initial findings in our recently developed co-culture system show a strong modulation of fibroblast responses by epithelial cells. This is seen in the release of cytokines interleukin (IL)-8 and IL-6 by fibroblasts in co-culture whiles there is a down-regulation in the mRNA expression levels of ECM molecules like α -SMA and fibronectin. Furthermore, the results indicate that differences may exist in epithelium-fibroblast crosstalk between health and COPD. However, the mechanisms underlying

this interaction and differences herein between health and COPD are still largely uncovered.

To understand the mechanism of the epithelial-fibroblast communication, plans involve conditioned medium experiments. Here, bronchial epithelium (16HBE cells or primary epithelial cells from control or COPD patients) will be grown to confluence and used to make conditioned medium with or without cigarette smoke extract pre-stimulation. **ELISAs will be performed on the conditioned medium from epithelial cells to determine the release of mediators like human IL-1α, IL-1β, IFN-γ &TNF-α**. All these mediators have been reported to be released by airway epithelium in COPD and may have similar effects on fibroblast as seen in our co-culture system. The conditioned medium from the epithelial cells will then be placed on fibroblasts with **various antagonists and neutralizing antibodies e.g. IL-1 receptor antagonist (IL-1RA), IL-1α, IL-1β, IFN-γ & TNF-α neutralizing antibodies**. The amount of IL-8 and IL-6 released and the protein expression levels of structural proteins α-SMA and fibronectin in fibroblasts will be measured to determine if blocking a particular mediator reverses the effects previously seen in our preliminary experiments. These inhibition and neutralization experiments will also be repeated in the co-culture model itself.

After identifying this mediator, recombinant human antibodies (e.g. IL-1α, IL-1β, IFN-γ, TNF-α) will be used to stain bronchial tissue from control donors and COPD patients to show how they are expressed in disease. Again, western blots (e.g. IL-1α, IL-1β, IFN-γ, TNF-α) will be performed on bronchial epithelium that has been pre-stimulated with CSE or not to assess the expression levels of the identified mediator. As a proof of concept, lung fibroblasts will be stimulated with recombinant human cytokines (e.g. IL-1α, IL-1β, IFN-γ & TNF-α) to show the effect of these mediators on fibroblast function.

This will throw more light on the differences between the aberrant epithelial-fibroblast cross-talk in COPD and suggest possible future targets for therapeutic interventions in COPD.