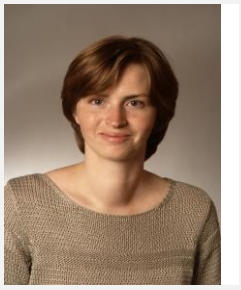
ImmunoTools *multiplex* Award 2014



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Influence of CCL18 on Alveolar Epithelial Cells Type II of fibrosis patients

Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease of unknown origin. The prognosis of IPF is poor and the treatment options are actually limited to one approved therapy, which only prevent the progression of the disease. Currently, lung transplantation is the only therapeutic option. IPF is characterized by accumulation of myofibroblasts in lung and formation of excess fibrous connective tissue. It is believed that an initial or repetitive injury of alveolar epithelial cells type I (AECI) results in an aberrant wound healing leading to the reorganization of the pulmonary tissue. Damaged AECI are normally replaced by alveolar epithelial cells type II (AECII) differentiation. However, in IPF one hypothesis is that AECII differentiate in presence of TGFβ to fibroblasts or myofibroblasts, a process which is termed as epithelial to mesenchymal transition (EMT) [1, 2].

Our data give evidence that CCL18, a chemokine found increased in serum of patients with pulmonary fibrosis, plays also an important role in IPF. CCL18 serum concentrations of patients with fibrosis are clearly correlated with their survival time [3].

Moreover, comparable to TGFβ CCL18 induces the production of matrix proteins such as collagen in fibroblasts, presuming a direct relationship between CCL18 and the progress of the fibrotic lung diseases [4].

In own experiments, we were able to demonstrate that isolated AECII stimulated with CCL18 can differentiate to cells with a myofiboroblast-like phenotype characterized by the increase of the typical mesenchymal marker αSMA.

The **Multiplex Array** would be an excellent tool to further analyse the participation of CCL18 in pulmonary fibrosis and the effect on AECII.

For the experiment we will isolate AECII from explanted lungs of IPF patients and from macroscopically tumour-free lung tissue of pulmonary resections of patients with lung cancer. This isolation procedure is well established in our lab [5]. The purity of the cells will be controlled by Papanicolau staining and flow cytometry.

$1X10^6$ AECII/ well from tumour-free lung tissue will be cultured in absence of CCL18 and AECII from IPF in absence or presence of CCL18 (100ng/ml) for 72h. To prevent the release of the cytokines, cells were incubated with 1µM Brefeldin 3h after stimulation. Comparison of non-fibrotic and fibrotic AECII will give us an overview of the basal differences of cytokine production and CD expression between these two groups. Additionally, the screening with the Multiplex Array of unstimulated versus CCL18 stimulated AECII will help us to further clarify the contribution of CCL18 in IPF and showing us on which cytokines and CDs we should paying more attention in future.

The Multiplex Award is limited to three samples, but we also interested in the analysis of CCL18 stimulated AECII isolated from tumour-free lung tissue and the consequences of blocking of CCL18 binding.

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2. Willis, B.C., et al., *Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis.* Am J Pathol, 2005. **166**(5): p. 1321-32.
3. Prasse, A., et al., *Serum CC-chemokine ligand 18 concentration predicts outcome in idiopathic pulmonary fibrosis.* Am J Respir Crit Care Med, 2009. **179**(8): p. 717-23.
4. Atamas, S.P., et al., *Pulmonary and activation-regulated chemokine stimulates collagen production in lung fibroblasts.* Am J Respir Cell Mol Biol, 2003. **29**(6): p. 743-9.
5. Hohne, K., et al., *Roflumilast-N-oxide induces surfactant protein expression in human alveolar epithelial cells type II.* PLoS One, 2012. **7**(7): p. e38369.