ImmunoTools *special* Award 2014

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The influence of macrophages and cytokines on pulmonary fibrosis

Fibrosis is considered as pathological replacement of functional tissue by connective tissue and is characterized by a dysregulated wound healing response. Well known initiators of fibrosis are viral infections, radiotherapy, chemotherapeutic drugs and aerosolized toxins. In contrast there are still unidentified trigger factors such as in idiopathic pulmonary fibrosis (IPF). The process of fibrosis often starts with alveolar epithelial cell lesion in pulmonary fibrosis. Subsequently inflammatory cells infiltrate the organ and stimulate fibroblast hyperplasia. Their maturation into myofibroblasts leads to an extensive collagen and extracellular matrix (ECM) deposition. This results in impaired tissue function due to destruction of the tissue architecture leading to an ineffective gas exchange in the lungs.

Suitable treatment options are scarce and the development of new therapies is needed. It is becoming increasingly apparent that innate immunity plays a central role in pulmonary fibrosis. Therefore we intent to elucidate the cytokines and chemokines involved in pulmunary fibrosis and to identify the cell types involved in this process in *in vivo* and *in vitro* studies.

Many cytokines and chemokines participate either in pro- or anti- fibrotic mechanisms. The two most important chemoattractants in fibrogenesis are the macrophage inflammatory protein 1α (MIP-1 α, CCL3) and the monocyte chemoattractant protein 1 (MCP-1, CCL2). Both chemokines recruit phagocytes and are produced by macrophages and epithelial cells. They most likely act pro- fibrotic through the stimulation of IL-4 and IL-13. In addition the chemokine receptors CXCR4 and CCR7 promote fibrosis through the recruitment of fibrocytes. The central effector molecule in fibrosis is IL-13, promotes the production of latent TGF-β1 in macrophages, as well as the synthesis of proteins which activate TGF-β. Blocking IL-13 depletes collagen deposition. In addition IL-4 is an important trigger of fibrosis and acts also on the IL-4 receptor on fibroblast subtypes, inducing the expression of collagen type I and III and fibronectin *in vitro.*

To study the impact of different kind of cytokines, we would like to stimulate alveolar macrophages with recombinant murine IL-4, IL-10, IL-13, IL-17 and M-CSF, cytokines known to be very important in the development of fibrosis. Upon stimulation we want to examine with qRT PCR the expression of the profibrotic cytokine TGF-β. Further we want to test different combinations of these cytokines and their effects on TGF-β expression. Additionally we want to co-culture alveolar macrophages pretreated with the recombinant

murine cytokines with either primary fibroblast or mouse embryonic fibroblast (MEF) cell line to identify how macrophages could directly influence fibroblast activation during pulmonary fibrosis.

Further we are interested if platelets can affect pulmonary fibrosis via acting on macrophages. To address this question we want to incubate activated platelets with macrophages and analyze the polarization state of the macrophages. To study the potential of platelets to influence macrophage activity we would like to treat macrophages with cytokines or growth factors released by platelets. In this context we especially want to focus on the role of CXCL4, RANTES, sCD40L, VEGF and PDGF-AA.

In the bleomycin induced mouse model of pulmonary fibrosis, not only macrophages but also neutrophils, T-cells and B-cells participate in the disease. To identify the various cell types in the bronchoalveolar lavage fluid of bleomycin treated lungs flow cytometric analysis is an optimal tool. To differentiate these cell types antibodies against mouse CD11b, CD3, CD4 and CD8 are quite useful and reliable markers.

To establish a link between mouse models of pulmonary fibrosis and the relevance for the human disease we additionally want to stimulate human monocytes with recombinant human IL-4, IL-10, IL-13, GM-CSF and M-CSF and subsequently examine TGF-β expression and the release of IL-6 and TNF-α via ELISA. Further we want to characterize the polarization of the monocytes via the flow cytometry antibodies anti-human CD14, CD16, CD80 and CD86. In addition we want to examine platelet cytokines, such as RANTES, sCD40L and PF4 on monocyte activation.

For the treatment of idiopathic lung fibrosis it would be most helpful to identify the specific triggers of these profibrotic cytokines to introduce possible new therapeutic approaches. The possibilities provided by ImmunoTools reagents would be great to study the fibrotic potential of cytokines in detail. Possibly combined blocking strategies of inflammatory cytokines have beneficial effects for patients suffering from pulmonary fibrosis.

ImmunoTools *special* AWARD for **Julia Kral** includes 25 reagents

FITC - conjugated anti-human CD86,

PE - conjugated anti-human CD14, CD80,

APC -conjugated anti-human CD16,

recombinant human cytokines rh GM-CSF, rh IL-4, rh IL-13, rh M-CSF, rh PF4v1/CXCL4V1, rh RANTES / CCL5, rh sCD40L / CD154,,

human IL-6 ELISA-set, human TNF alpha ELISA set

FITC - conjugated anti-mouse CD3e,

PE - conjugated anti-mouse CD8a, CD11b,

APC -conjugated anti-human CD4,

recombinant mouse cytokines rm IL-4, rm IL-10, rm IL-13, rm IL-17A, rm M-CSF, rm PDGF-AA, rm RANTES / CCL5, rm sCD40L / CD154

DETAILS