ImmunoTools *special* Award 2022

Mugdha Joglekar, MSc., PhD-student

Supervisors: Prof. Dr. J.K. Burgess, Dr. S.D. Pouwels

Department of Pathology and Medical Biology, University Medical Center Groningen, Experimental Pulmonology and Inflammation Research lab (EXPIRE), Hanzeplein 1, 9713 GZ Groningen, The Netherlands

Interplay between the extracellular matrix and neutrophils in chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterized by chronic lung inflammation mediated by pro-inflammatory cytokines and enzymes secreted by resident cells of the lungs and infiltrating immune cells. Neutrophils have been shown to play a central role in these inflammatory processes, with sputum and blood neutrophilia being key features of this disease. Upon recruitment from blood, neutrophils migrate to the site of inflammation and secrete proteases to remodel the extracellular matrix (ECM), the scaffold that supports cells and provides structure and rigidity to the lungs. The ECM not only behaves as a physical scaffold but also regulates cell proliferation, migration, and differentiation by providing crucial biochemical and biomechanical cues. Consequently, it regulates neutrophil function and fate in healthy and disease conditions. Previously, neutrophil elastase (NE), C-X-C Motif Chemokine Ligand 8 (CXCL8), and matrix metalloproteinase (MMP) 9 were found elevated in sputum of COPD patients compared to controls. Moreover, neutrophils isolated from COPD patients were reported to have increased speed but reduced directionality of migration. Interestingly, matrix proteins such as mindin and lumican have been demonstrated to be fundamental in neutrophil recruitment to the site of inflammation. Additionally, laminin was shown to induce neutrophil chemotaxis. A novel concept is that the ECM can direct neutrophil functioning by regulation of neutrophil apoptosis. However, these processes are less understood as conflicting studies have reported decreased rates of apoptosis of neutrophils isolated from peripheral blood of COPD patients, but also a higher ratio of apoptotic neutrophils in the sputum of COPD patients compared to healthy subjects. Increasing evidence, including COPD and non-COPD patient serum samples analyzed in our study, points towards the dysregulation of lung ECM composition and turnover in COPD compared to healthy conditions. Therefore, we hypothesize that the interplay between neutrophils and lung ECM in COPD generates a feedforward loop prolonging the inflammatory responses. In our study, we aim to further investigate this neutrophil-ECM interplay in COPD.

To examine the influence of the ECM on neutrophil migration, inflammatory responses, and survival we will isolate neutrophils from venous blood of healthy human donors, followed by

evaluation of their purity and activation state by quantifying the expression of CD11b, CD16, CD62L, and CD66b using flow cytometry. Neutrophil migration will be visualized using Ibidi chemotaxis slides in the presence of a CXCL8 gradient. For studying inflammatory responses and survival, neutrophils will be seeded on ECM deposited by human lung fibroblasts derived from donors with and without COPD. The secretome will be investigated in supernatant after 8-24 hours by measuring NE, MMP9, IL-1β, IL-6, CXCL8, IL-10, IL-12, dsDNA, and MPO using ELISAs and flow cytometry. Additionally, neutrophil survival in the presence of non-COPD and COPD ECM will be compared by flow cytometry using FITC conjugated Annexin V-PI.

Lung fibroblasts are major producers of ECM in the human lungs. To investigate the contribution of neutrophils in perpetuating ECM remodeling and disruption in COPD, neutrophils will be cocultured with COPD and non-COPD fibroblasts. The fibroblasts will be investigated for their proliferation using Ki-67 staining and MTT assay and wound healing responses including collagen deposition by Sirius Red staining and FGF2, PDGF, TGF-β1, VEGF, CTGF secretion by ELISAs in the presence of neutrophils. Moreover, after 8-24 hours of coculture, fibroblasts will be collected and investigated for gene expression of ECM proteins including collagen, elastin, decorin, and versican and inflammatory factors IL-6, CXCL8, INFγ, and TNF-α. Simultaneously, the secretome will also be collected and analyzed for the same inflammatory factors (IL-6, CXCL8, INFγ, and TNF-α) using ELISAs.

COPD currently is the third leading cause of mortality worldwide and remains incurable. Neutrophilic inflammation, a prominent feature of COPD, does not respond to current treatment options including corticosteroids which is reflected in disease progression and mortality. Hence, there is an urgent need for effective anti-neutrophilic therapies for COPD. The ImmunoTools special Award will enable us to answer some of our research questions to gain a better understanding of pathobiology and possibly shed light on new research targets for therapeutic interventions for COPD.

ImmunoTools *special* AWARD for **Mugdha Joglekar** includes 10 reagents

FITC - conjugated Annexin V

recombinant human rh IL-8/rh CXCL8

human ELISA-set (for one 96 plate): human IL-6, human IL-8 (each four reagents)

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