The role of neutrophils and their proteases in the pathology of allergic airway disease

Final Report Summary - NEUPROTALL (The role of neutrophils and their proteases in the pathology of allergic airway disease)

The role of neutrophils and their proteases in the pathology of allergic airway disease (NEUPROTALL).

This project has provided a unique framework to define the role of neutrophils and their proteases in directing the cellular inflammation and pulmonary remodelling in a clinically relevant model of allergen induced airway disease. Furthermore, this project has expanded our knowledge of the role of neutrophils in modulating the immune response against respiratory viral infections, which are known to be related to asthma.

This project had the following specific aims: 1. To define the contribution of neutrophils to the development of the chronic inflammation and structural changes observed within the lungs of mice exposed to house dust mite (HDM); 2. To determine to what extent neutrophils are responsible for the pulmonary protease imbalance in the allergic lung and to define the roles of these proteases and the matrikines they produce in the pathology of allergic asthma; 3. To determine how neutrophils alter the homeostatic regulatory pathways within the lung and ensuing alveolar macrophage responsiveness to allergen. We hypothesised that neutrophils and their proteases are important in the initiation and maintenance of the cellular immune response to allergen and contribute to the ensuing tissue destruction and remodelling.

To develop this project we have used an established murine model of HDM-induced asthma, as well as relevant murine models of respiratory infections (Influenza virus, respiratory syncytial virus (RSV), Haemophilus influenzae b (Hib), Streptococcus pneumonia, and the toll-like receptor agonist lipopolysaccharide (LPS)). To assess the role of neutrophils in these models, mice were treated with either a control (2A3) or a neutrophil specific depleting (1A8) antibody and were exposed to the allergen or to the different infectious agents. Downstream tissue processing and analysis was performed at various time points thereafter. Multiple techniques have been used throughout this project. Complex multi-parameter flow cytometry has been utilized to assess the inflammatory response. Analysis of damage markers, cytokines, chemokines and proteases of interest has been done both at the protein (ELISA, Western blot, zymograms,
immunohistochemistry and immunofluorescence) and RNA level (qPCR). In addition, alveolar macrophages and neutrophils have been sorted (using MACS technology), and cell culture systems have been developed to dissect the mechanisms in in vitro settings that complement the in vivo models. Furthermore, the use of different inhibitors as well as wild-type and specific knock-out mice in our in vivo models has further contributed to dissect the mechanisms involved.

The research work performed within this project has led to the following main results:

Aim 1. In our HDM-induced allergic airway disease murine model, neutrophil depletion surprisingly yields an augmented Th2 inflammation in the lung and airways after three weeks of HDM exposure. This exacerbated Th2 inflammation in neutrophil depleted animals is due to a preceding increase in Th2 cytokines, IL-4, IL-5 and IL-13, after one week of HDM exposure that is derived from a novel pathological monocyte population. These monocytes are augmented in neutrophil depleted animals as a result of an increase in progenitor pools within the bone marrow. Thus, neutrophil-depletion exacerbates allergic Th2 inflammation due to a previously unrecognized capacity of neutrophils to regulate aspects of myelopoiesis.

Aim 2. In our HDM-induced allergic airway disease murine model, the levels of proteases matrix metallopeptidase-9 (MMP-9), myeloperoxidase (MPO) and neutrophil elastase (NE) were elevated and were all largely ablated with neutrophil depletion. Intriguingly, the predominantly macrophage protease MMP-12 was also elevated and we have shown that this is dependent on neutrophil infiltrate. We have demonstrated that MMP-12 peaks at 3 weeks of HDM, with a higher expression in Balb/c mice, compared to other strains, which show a more neutrophilic phenotype of the disease. Furthermore, MMP-12 levels seem to be IL-13 dependent. We are currently dissecting the role of MMP-12 to the development of the chronic inflammation and structural changes observed within the lungs of mice exposed to HDM and determining the impact of therapeutic manipulation of MMP-12 in allergic airways disease.

Aim 3. We have addressed how neutrophils alter the homeostatic regulatory pathways within the lung and ensuing alveolar macrophage responsiveness in a short-term model of primary exposure to HDM and in different respiratory infectious models (Influenza, respiratory Syncitial virus (RSV), bacterial infections and TLR agonists) using a neutrophil depleting strategy. We found a striking phenotype during respiratory viral infection with a profound effect of neutrophils on alveolar macrophage activation. In our model, influenza elicited a robust release of IL-1β in the airways at 24 hours post-infection that correlated with neutrophil infiltrate and was completely ablated by neutrophil depletion. Viral infection triggered the expression of the NLRP3 inflammasome and pro-IL-1β in alveolar macrophages. However, subsequent activation of the inflammasome complex and release of mature IL-1β from alveolar macrophages was critically dependent upon the provision of a secondary signal from infiltrating neutrophils. Thus, neutrophils are critical for the activation of the NLRP3 inflammasome in alveolar macrophages during respiratory viral infection.

The development of this project has led to an abundance of significant findings, which have been disseminated internally, at Department Meetings, and externally. In this sense, several manuscripts are currently being prepared for submission or have been recently submitted to be considered for publication in leading high impact scientific journals. These findings have also been presented at the international Keystone Symposia “Myeloid Cells”. We aim to further disseminate these results at the BSI/NNVV1 Annual Congress 2016, where an abstract has been submitted to be considered for presentation.

In conclusion, this project has studied in depth the role that neutrophils play in modulating the immune response in various respiratory diseases and has contributed to the scientific advance in this field. In this respect, we have described a previously unrecognized capacity of neutrophils to regulate various aspects of myelopoiesis, with a profound effect on allergic airway disease. Furthermore, we have demonstrated a novel role for neutrophils in activating alveolar macrophages and enabling them to respond more robustly against respiratory viral infections. Moreover, this project has provided interesting results regarding the role of MMP-12 in asthma that we will further pursue in upcoming projects. Thus, the results of this project provide a novel insight into the role of neutrophils and ascribe new mechanisms that these cells undertake during asthma and respiratory viral infections. Furthermore, due to the broad scope of our findings, we consider that their impact will not be limited to these diseases.

In sum, this project has contributed to the advance of the scientific knowledge and understanding of neutrophils and their role in respiratory diseases and has highlighted new pathways and targets that will contribute to future research studies that lead to drug design and novel therapies for disease, contributing to decrease the impact of these diseases in society.