Decoding the immune and structural landscapes of the prenatal and emphysematous lung at the single-cell level

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Appendices

Summary in English

Introduction

The lungs enables respiration, through a complex structure comprising the trachea, bronchi, bronchioles and facilitating gas exchange in the alveoli. Epithelial cells and immune cells within the lung play a vital role in defending against inhaled pathogens and maintaining homeostasis. However, factors such as congenital lung malformations, cigarette smoking, air pollution, and recurrent infections can lead to chronic respiratory diseases like chronic obstructive pulmonary disease (COPD). COPD is the third largest cause of mortality worldwide. In the majority of COPD patients, progressive inflammation causes irreversible alveolar tissue damage (emphysema) leading to impaired gas exchange and loss of lung function, which severely impacts the quality of life of patients with emphysema. Therefore, a better understanding of the interactions between structural- and immune cells in developing, healthy and diseased lungs, may provide clues to develop strategies to enhance regeneration processes of diverse types of lung cells in emphysema. In addition, identifying the dysregulated structural and immune cell compartments in emphysema can contribute to a better understanding of emphysema development, which also benefits development of treatment strategies based on the dysregulated cell profiles.

The co-development of structural and immune cells in the human fetal lung

In **Chapter 2**, we illustrated the dynamic development and cellular interactions of structural and immune cell components in the human fetal lung throughout the pseudoglandular and canalicular stages using imaging mass cytometry. Here we mapped the developing structural components in the human fetal lung with specific marker phenotypes, and observed distinct immune compartments consisting mainly of myeloid cells. Whereas $CD206^{+}CD68^{+}$ macrophages were present already at the early pseudoglandular stage, $HLA\text{-}DR^{+}$ myeloid cells emerged at the end of pseudoglandular stage. Moreover, cellular interaction analyses revealed an accumulation of $HLA\text{-}DR^{+}$ cells near the $KRT8^{+}EPCAM^{+}$ structural regions, suggestive of their involvement in the maturation of epithelial lung structures. We provided detailed information on the localization of the developing cells and visualized important cellular interactions, which

contributes to the knowledge on normal lung development. Ultimately this information may be utilized to devise strategies for tissue regeneration in chronic lung diseases like emphysema.

Loss of pulmonary endothelial cells in emphysema impairs support of human alveolar epithelial cell growth

Cellular interactions and signaling in the alveolar niche are altered in patients with COPD, resulting in impaired repair responses and alveolar tissue loss. In **Chapter 3**, we showed that the expression of microvascular $CD31^+$ endothelial cells was significantly lower in the alveolar compartment in emphysema, and further demonstrated a supportive role of human endothelial cells in the proliferation of alveolar type 2 cells and the formation of lung organoids. Additionally, we provided evidence that exposure to cigarette smoke extract impairs this supportive function. Our data therefore suggest that a combination of both a lower number of microvascular endothelial cells and endothelial dysfunction contributes to impaired alveolar epithelial regeneration. Unraveling this supportive role of microvascular endothelial cells in alveolar regeneration provides a potential target for preventive and regenerative medicine approaches in emphysema.

Emphysema-associated changes in the pulmonary immune system

Further, we provided an overview analysis of the pulmonary immune system in emphysema and control lung tissues using single-cell mass cytometry (CyTOF) in **Chapter 4**, to understand the lung microenvironment in emphysema. We discovered emphysema-associated immune cell subsets and provided evidence for their function within the emphysema microenvironment. We observed higher percentages of central memory CD4 T cells and central memory CD8 T cells in emphysema, which exhibited an IFN-γ response upon anti-CD3 and anti-CD28 activation. Proportions of $CD1c^+$ dendritic cells expressing migratory and costimulatory markers, were higher in emphysema. Moreover, we confirmed these emphysema-associated immune subsets in historically stored formalin-fixed paraffin-embedded and snap frozen lung tissue blocks, and visualized specific immune cellular interactions *in situ* by imaging mass cytometry (IMC). IMC results showed increased spatial colocalization of $CD1c^+$ dendritic cells and CD8 T cells in emphysema *in situ*. These data contribute to a better understanding of the pathogenesis of emphysema and highlight the feasibility of

interrogating the immune cell signature using CyTOF and IMC in human lung tissue.

Pulmonary and systemic immune profiles following lung volume reduction surgery and allogeneic mesenchymal stromal cell treatment in emphysema

Emphysema is characterized by progressive inflammation, and treatment options that halt or reverse emphysema are limited. Preclinical studies suggest that lung volume reduction surgery (LVRS) and mesenchymal stromal cell (MSC) treatment may dampen inflammation. In **Chapter 5**, we described a clinical study in emphysema patients undergoing lung volume reduction surgery and receiving bone marrow-derived MSC (BM-MSC) or placebo infusion, and we investigated the effect of BM-MSC and two times LVRS on circulating and pulmonary immune cell profiles in these emphysema patients. We observed that BM-MSC treatment did not affect the immune profiles. However, the proportions of circulating lymphocytes, including central memory CD4, regulatory, effector memory CD8 and γδ T cells were higher, whereas myeloid cell percentages were lower in second LVRS (L2) compared to the first LVRS (L1), irrespective of BM-MSC or placebo treatment. Moreover, pseudotime analysis revealed potential migration of blood-derived monocyte subpopulations to emphysematous lung tissue, potentially contributing to resident macrophage subpopulations. In total, there were no effects of BM-MSC treatment on immune profiles in patients with severe emphysema. However, we observed several changes in circulating and pulmonary immune cells upon LVRS, suggesting the induction of anti-inflammatory responses that may be needed for repair processes.

Conclusion

In summary, we provide profiles of the developing embryonic lung immune cells and epithelial cells in the human fetal lung, and provided comprehensive information on the immune profiles related to the pathophysiology of emphysema, as well as the consequence of LVRS and BM-MSC treatment. The findings in this thesis will facilitate further mechanistic studies of immune cells and structural cells in emphysema. We also anticipate that these previously unrecognized immune cell populations and damaged lung structural cells may offer insights into potential treatment strategies.