Immune Phenotyping of Bronchoalveolar Lavage in Children with Cystic Fibrosis

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Cystic Fibrosis (CF) is thought to affect around 70,000 people worldwide and current estimated life expectancy is 44 years. Lung disease, the major source of morbidity and mortality in CF, is multifactorial and characterised by altered airway surface liquid which leads to impaired mucociliary clearance and a pre-disposition to infection. The combination of recurrent infection and inflammation further progresses disease severity. There is large variation in the trajectory and severity of lung disease in CF, and accurate prediction of severity would address 8 of the top 10 international research priorities in CF. Despite this need, extensive work has thus far failed to identify robust and well validated markers that can be used in clinical practice. Bronchoalveolar lavage (BAL) is a logical source to examine for potential predictors of lung disease severity as it samples the tissue of interest and is already collected as part of the clinical management of patients with CF. The primary purpose of this study was to develop a flow cytometry-based immune phenotyping panel for cryopreserved BAL collected from children with CF and investigate early life immune determinants of lung disease severity. Using the following surface markers, we were successfully able to phenotype, quantify and purify the four most abundant cell populations in BAL from children with CF: alveolar macrophages (CD45⁺CD206⁺CD15⁺SSC<sub>high</sub>), granulocytes (CD45⁺CD206⁻CD15⁺SSC<sub>high</sub>), total lymphocytes (CD45⁺SSC<sub>low</sub>) and alveolar epithelial cells (CD45⁻EpCAM⁺). Additional markers for cells of lymphoid (CD3, CD4, CD8) and myeloid (CD11c, HLA-DR, CD14, CD16) lineage permitted further investigation of the immune cell composition of BAL. We additionally showed that structural lung disease in the first five years of life (determined by evidence of bronchiectasis on CT scan at time of BAL collection) is associated with increased granulocyte recruitment and an alerted inflammatory cytokine milieu in the BAL. This protocol has multiple applications and could be used in larger studies to assess predictors of lung disease severity over the lifecourse of CF that could readily be used in clinical practice.