Organ rejection after lung transplantation remains a significant clinical problem. Chronic rejection is the major contributor to morbidity and mortality following lung transplantation; the median survival is only 5.6 years. A third of all patients is treated for an episode of acute rejection within the first year after transplant. Recurrent episodes of acute rejection have been identified as a major risk factor for developing chronic rejection.

Currently, we cannot identify and treat patient at risk for rejection; therefore patient’s immunosuppressive therapy is typically intensified during episodes of acute rejection; however direct testing of the efficacy of immunosuppression is not available.

To quantify immune response and assess individual rejection potential, new cellular assays are needed. Macrophages play a critical role in maintaining immune homeostasis; they have both pro- and anti-inflammatory properties. The complex interactions between alveolar macrophages, lung epithelial cells and immune cells are poorly understood. Knowing particular changes in the phenotype of alveolar macrophage after lung transplantation and how these changes correlate with activation of other immune cells may result in new therapies to modulate inflammation and limit allograft rejection.

Hypothesis
We hypothesize that unique phenotypic changes in alveolar macrophages and blood cells precede clinical evidence of rejection.

Study objectives
To address this hypothesis we propose to: 1) Identify phenotypic and functional changes in immune cells residing within the lung that correlate and precede the clinical evidence of lung rejection. 2) Assess the efficacy of immunosuppression utilizing mass cytometric profiling of blood lymphocytes. Based on our findings we aim to develop a panel of markers associated with rejection that could be used as a diagnostic tool for immune monitoring after receiving lung transplant.

Outline
At the University of Virginia all lung transplant recipients are followed closely with lung function testing at every visit and surveillance bronchoscopies at 1, 3, 6, 9, and 12 months post transplant and whenever clinical concern for rejection occurs. We will analyze bronchoalveolar lavage (BAL) fluid and paired peripheral blood samples from these time points, which will allow us to longitudinally follow changes in BAL and blood immune cells and correlate the findings with the clinical condition. Over one year we anticipate collecting about 100 data points from 20 patients. Given that 30% of them have one episode of acute rejection within the first year after transplantation, we estimate to see 6-10 episodes of rejection per year.
To comprehensively profile immune cell populations we will utilize mass cytometry which combines high-speed single cell analysis with mass spectrometry allowing to analyze over 100 metal-probes directed to target proteins at the same time. Furthermore we will use multiplex assays for cytokine and chemokine analysis in the BAL-fluid as well as in serum.

BAL-supernatant and serum will be tested for various cytokines and chemokines using multiplex assays. BAL cells and peripheral blood cells will be stained with metal-labeled antibodies for further profiling using mass cytometry.

The antibody composition will include CD3, CD4, CD8a, CD11c, CD14, CD16, CD19, CD20, CD27, CD45, CD45-RA, CD61, CD66, HLA-DR, EMR1, Siglec-8 to differentiate T- and B-lymphocytes, monocytes, dendritic cells, eosinophils, and neutrophils.

As macrophages change their functional phenotype in response to the microenvironment we will focus specifically on profiling alveolar macrophages. We will further characterize them by the expression of CCR2, CX3CR1, CD64, CD62L, CD80, CD86, CD163, CD200, CD206, IL1RI and IL10R, IL13R, TGFbeta-R, TLR1, TLR2, TLR4, iNOS, INFgamma, TNFalpha, IL1beta, IL6, IL12, IL23, IL10, TGFbeta, CCL1, CCL2, CCL5, VEGF, COX-2, SOC3, JAK1, STAT3, JNK, ERK, IkappaB, RHOA, SMAD.

In the peripheral blood cells we will further focus on subtypes of effector and regulatory T-cells, utilizing the following markers: CD2, CD5, CD7, CD9, CD11a, CD25, CD28, CD44, CD49d, CD57, CD127, CD69, CD161, CCR4, CCR5, CCR7, CXCR3, FOXP3, IL10, IL4, IL5, IL12, IL17, Granzyme B, STAT1, STAT4, Tbet, Gata-3, INFgamma. The BAL fluid and the serum will be tested for TH1 and TH2 cytokines and chemokines.

Mass cytometry data will be correlated with the clinical condition of each patient. We will use our data to longitudinally follow single patients, but also to compare cells from patients with and without evidence of rejection.

Outcomes and Innovation

We aim to identify distinct changes in phenotype, function and composition of immune cells in the BAL and blood that correlate with the development of acute rejection. This work is innovative in several ways. Conceptually, we expect to develop a panel of biomarkers for patients at risk developing acute rejection. It is conceivable that macrophage markers and specific immune cell combinations will be informative indicators of transplant condition. With novel flow cytometry technologies, such as CyTOF, capable of analyzing dozens of markers in a single sample, a panel of disease state-specific markers can be developed that serve to predict episodes of acute rejection. Moreover, the results of this study may help better analyze the efficacy of immunosuppression. To the best of our knowledge, this is the first study employing mass cytometry in lung transplant recipients.

The proposed studies will make extensive use of BD Biosciences reagents, specifically primary antibodies directed to cell surface markers and signaling molecules, other flow cytometry-related reagents, as well as BD’s cytometric bead assays.