**Resident Scholarly Project**
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**Faculty Mentor**: Aliva De, MD
**Title of Project**: Airway Inflammation in Sickle Cell Disease

**Study Purpose and Rationale**
Respiratory complications (affecting over 50% patients with SCD) are the leading cause of morbidity and death in SCD. Acute chest syndrome is an acute complication of SCD, and one of the leading causes of death in SCD. Recurrent episodes of acute chest syndrome may compromise future lung function. These pulmonary abnormalities involve complex inflammatory pathways contributing to both obstructive (reversible/non-reversible) and restrictive disease that is not well characterized. Airway inflammation in SCD is generally considered to be due to asthma. For this project, we plan to determine baseline T-helper cell, monocytic, and leukotriene inflammatory pathways in sickle cell disease patients using a repository of samples from a previously conducted clinical trial (ViDAS clinical trial- supported by Grant R01 FD003894 from the US Food and Drug Administration Orphan Product Development), to test the hypothesis that patients with baseline history of acute chest syndrome and pulmonary dysfunction have a predominant T-helper 1 and monocytic pattern of inflammation that will correlate with their pulmonary function test deficits. We further plan to investigate changes in the inflammatory markers and relationship with respiratory outcomes at 2 years following vitamin D therapy.

**Study Aim and Hypothesis**
The overall goal of this project is to improve therapy for pulmonary dysfunction in children with sickle cell disease (SCD) by characterizing inflammatory phenotypes. Airway inflammation in SCD is generally considered to be due to asthma. However, initial studies performed by Dr. De among SCD children with pulmonary dysfunction have found distinct inflammatory phenotypes with elevated T-helper 1, T-helper 2 and monocytic markers, distinct from classic allergic asthma (elevated T-helper 2 only). Accordingly, both atopic and non-atopic immune responses can mediate SCD airway inflammation. Furthermore, murine studies of allergic sensitization in SCD have shown non-T-helper 2 dominant patterns of inflammatory response to allergic stimulation. Vitamin D therapy has been investigated and is speculated to have a beneficial effect on reducing asthma exacerbations and respiratory infections. The postulated mechanism of action is through T lymphocyte mediated immune–modulation. A study conducted at the Sickle Cell clinic at Morgan Stanley Children's Hospital, Columbia University Medical Center, investigated the effects of monthly standard dose and high dose Vitamin D3 for 24 months on respiratory event outcomes (ViDAS clinical trial- supported by Grant R01 FD003894 from the US Food and Drug Administration Orphan Product Development). A repository of serum samples from 62 patients was stored for future analyses. The study demonstrated a decrease in respiratory events during 2 years of treatment, with no differences between the 2 treatment groups. For the current project, we plan to determine baseline T-helper cell, monocytic, and leukotriene inflammatory pathways in sickle cell disease patients using the repository of samples from the afore-mentioned study to test the hypothesis that patients with baseline history of acute chest syndrome and pulmonary

dysfunction have a predominant T-helper 1 and monocytic pattern of inflammation that will correlate with their pulmonary function test deficits. We further plan to investigate changes in the inflammatory markers and relationship with

**Methods**

Using a previous reservoir of serum samples that were collected as part of the "Vitamin D for Sickle-cell Respiratory Complications" Phase-2 clinical trial, we will be able to characterize the baseline inflammatory characteristics of the sickle cell population enrolled in this study at Morgan Stanley Children's Hospital, Columbia University Irving Medical Center, NY. There were 62 patients enrolled and randomized in this study to receive Vitamin D therapy. About half of these patients were known to have history of acute chest syndrome, asthma and/or abnormal pulmonary function tests with obstructive and/or restrictive lung function. About a third of the patients are expected to have allergies.

Stored serum samples from the "Vitamin D for Sickle-cell Respiratory Complications" Phase-2 clinical trial will be accessed, PFT data and clinical information for the patients in the clinical trial will be abstracted and the following tests will be conducted.

Serum Analysis of Cytokines: 12 serum cytokines will be quantified using the Multiplex technology, including those associated with T-helper 1/T-helper 17 inflammation (IFN-, TNF, IL-2, IL-8, IL-17, IP-10), T-helper 2 inflammation (IL-4, IL-5, IL-9, IL-13), and monocyte activation (MCP-1, and IL-6) will be quantified using the Luminex technology.

Briefly, 25ul of serum in duplicate will be incubated with magnetic beads coated with specific antibodies to a cytokine/chemokine for 30 minutes followed by incubation with a second antibody labeled with the fluorescent probe. The samples will be analyzed using Luminex 200 analyzer (Luminex Corporation, Austin, TX, USA) which quantifies each cytokine/chemokine by laser detection of fluorescence of the second antibody.

Data analysis will be performed using Luminex xPONENT3.1 software and Milliplex Analyst 5.1. Results will be reported in pg/mL. Serum Analysis of Cysteinyl Leukotrienes Serum Cysteinyl leukotrienes LTB4 will be quantified using commercial ELISA kits. Assessment of atopic status will be from medical records and pre-study screening questionnaire. Pulmonary Function Tests (PFTs) Results of PFTs performed during the study will be obtained and categorized into obstructive, restrictive and diffusion defects.

Demographic information such as age, gender, ethnicity, birth history, medical history including complications of SCD such as acute chest syndrome, VOC, etc, personal and family history of asthma, atopy, seasonal allergies, use of medications including inhaled corticosteroids, bronchodilators, hydroxyurea, analgesics for sickle cell pain, need for transfusion, number of hospitalizations or ER visits, surgical history, etc. will be collected.

Diagnostic test information such as hemoglobin electrophoresis, echocardiogram, trans-cranial Doppler, and overnight polysomnogram will be collected. Markers of hemolysis including baseline reticulocyte count and LDH level for SCD patients as well as baseline CBC results will be collected. We will further investigate changes in inflammatory markers at 2 years of the clinical trial to test the hypothesis that decrease in respiratory events were secondary to reduced cytokine expression. These investigations will provide important new clinical information and guide future therapeutic interventions in SCD.

**Study Drugs or Devices**
N/A

**Study Instruments**
N/A

**Study Subjects**
This study will enroll children with SCD with history of ACS who were included in the ViDAS trial with existing PFT and serum samples available.

Current age of the child can vary from 0-21 years.

**Confidentiality of Study Data**
Unique identifiers will be used for all data collected for the study. The samples sent for sequencing will be labeled with the unique study code and the individuals in the sequencing laboratory will not have access to the link to the subject identity.

**Potential Benefits**
The results obtained from this study can help us better understand the types of inflammation associated with acute chest syndrome and allergies in sickle cell disease patients and their relationships to lung function. This can potentially benefit sickle cell patients by advancing knowledge in this field and identifying future targets of therapy for these patients

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