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TITLE: Herpesviruses and Immune Dysregulation in Pulmonary Fibrosis

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CONTRACTING ORGANIZATION: Vanderbilt University Medical Center, Nashville, TN

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14. ABSTRACT:

The objective of these studies is to determine the role of human herpesviruses and antiherpesvirus immunity in the development of idiopathic pulmonary fibrosis. The first aim of the study is to collect peripheral blood and broncholalveolar lavage fluid from patients with IPF or other ILDs undergoing clinical bronchoscopy, quantify viral load and perform immunophenotyping of circulating and BAL immune cells. The second aim is to use a model of genetic risk for IPF to investigate the mechanisms of herpesvirus-driven experimental fibrosis.

15. SUBJECT TERMS: Idiopathic pulmonary fibrosis, Interstitial lung disease, Bronchoalveolar lavage, Alveolar epithelial cells, Cytomegalovirus, Epstein-Barr virus, Endoplasmic reticulum stress, T lymphocytes, Programmed Death-1

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Table of Contents

Page

1. INTRODUCTION:

It is widely recognized that recurrent injury to alveolar epithelial cells (AECs) initiates the process of progressive fibrotic remodeling in Idiopathic Pulmonary Fibrosis (IPF). Although the environmental factors that cause repetitive AEC injury in IPF are not well understood, both observational and experimental data support a role for herpesvirus reactivation in this process. Our preliminary data show that cytomegalovirus (CMV) and Epstein-Barr virus (EBV) antigens can be detected in AECs lining areas of fibrosis in the majority of IPF patients, whereas herpesvirus protein expression is not found in AECs in normal lungs. Herpesvirus antigens in AECs from IPF patients co-localize with markers of endoplasmic reticulum (ER) stress, which is a nearly universal finding in IPF lungs and contributes to AEC dysfunction and apoptosis. Our studies test the hypothesis that: 1) re-activation of latent herpesviruses, particularly CMV and EBV, in dysfunctional type II AECs contributes to a recurrent injury-repair cycle that drives progressive fibrosis in patients with IPF, 2) targeted anti-herpesvirus therapy or interventions to restore immunosurveillance will limit progression of lung fibrosis. Specific aims of this project are: 1) to determine viral load and immune response to herpesviruses in pulmonary fibrosis patients and 2) to investigate the mechanisms of herpesvirus-induced lung fibrosis in mice with AEC dysfunction.

2. KEYWORDS:

Idiopathic Pulmonary Fibrosis
Interstitial lung disease
Bronchoalveolar lavage
Alveolar epithelial cells
Cytomegalovirus
Epstein-Barr virus
Endoplasmic reticulum stress
T lymphocytes
Programmed Death-1

3. ACCOMPLISHMENTS:

What were the major goals of the project?

This project consists of 2 specific aims:

Aim 1. To determine viral load and immune response to herpesviruses in pulmonary fibrosis patients.

Major task 1: Enroll 100 subjects with pulmonary fibrosis in prospective cohort study.

Major task 2: Determine whether herpesvirus loads differ in IPF lungs compared to controls.

Major task 3: Define the herpesvirus-related T-cell phenotypes.

Aim 2. To investigate the mechanisms of herpesvirus-induced lung fibrosis in mice with AEC dysfunction.

Major task 1: Determine whether alveolar epithelial endoplasmic reticulum stress affects quantitative or functional T cell responses to herpesvirus infection.

Major task 2: Determine whether antiviral treatment can prevent or reverse T-cell exhaustion.
Major task 3: Test whether therapies designed to prevent or reverse T-cell exhaustion can enhance viral clearance and prevent lung fibrosis.

- **What was accomplished under these goals?**

Aim 1:

Major task 1: Enroll 100 subjects with pulmonary fibrosis in prospective cohort study.

During the last year, we achieved our enrollment goal of 100 subjects as shown in **Table 1**. The majority are male and the mean age at enrollment is 64.3 years. Based on multi-disciplinary conference evaluation, 40% were diagnosed as UIP/IPF, while the remainder have other ILD diagnoses, including chronic hypersensitivity pneumonitis, connective tissue disease-related ILD, and nonspecific interstitial pneumonitis. We are continuing to collect follow-up data, including PFTs and blood samples. All clinical data are entered into a secure Redcap database.

Table 1. Characteristics of Enrolled Subjects

	n=100
Age	64.33 (11.31)
Sex (female)	46
Pulmonary Function Tests	
FVC%	70.55 (19.49)
FEV1%	75.65 (20.25)
TLC%	71.35 (19.05)
DLCO%	48.21 (18.54)

Major task 2: Determine whether herpesvirus loads differ in IPF lungs compared to controls.

We are continuing to collect BAL samples and plan to batch samples for measuring viral load in the lungs. CMV and EBV serology testing has been done on samples collected to date. Table 2 summarizes CMV and EBV status of patients included in this study based on the data generated so far. Serology testing on the remaining subjects will be performed during the next year. We have performed tests for viral load quantification on BAL samples using droplet digital PCR (ddPCR) or regular PCR reaction. Although we have used different methods for DNA extraction, we have some difficulty with reproducibility of viral quantification in BAL. Our plan is to continue investigating the presence of lytic virus in BAL using different probes. Alternatively, we could determine virus quantification using conventional cell culture.

Table 2. CMV and EBV SEROLOGY

CMV+ EBV+ Total 28
9 Females (Average age: 68)
19 Males (Average age: 67)
EBV+ Total 12
4 Females (Average age: 66)
8 Males (Average age: 67)
CMV- EBV- Total 3
1 Female (Age: 61)
2 Males (Average age: 73)

Major task 3: Define the herpesvirus-related T-cell phenotypes.

To analyze immune and inflammatory cells in the lungs of patients with IPF and non-diseased control subjects, we obtained explanted lungs at the time of lung transplantation or organ procurement from 12 patients with IPF and 15 organ donors whose lungs were declined for transplantation (**Figure 1A**). After enzymatic digestion, single-cell suspensions were interrogated using a 31-parameter mass-cytometry (CyTOF) panel (**Table 2**). Joint analysis of these 27 samples was performed to generate viSNE maps among $CD45^+$ leukocytes to identify distinct cell populations (**Figure 1B**). Based on expression of canonical markers, we labeled T cells (CD3), B cells (CD19), myeloid cells (CD11b, CD11c, HLADR and CCR2), neutrophils (CD24) and NK cells (CD16, CD38) (**Figure 1B-C**) Except for a reduction in NK cells, no substantive shift in major immune/inflammatory cell types was identified in IPF lungs compared to controls (**Figure 1C**).

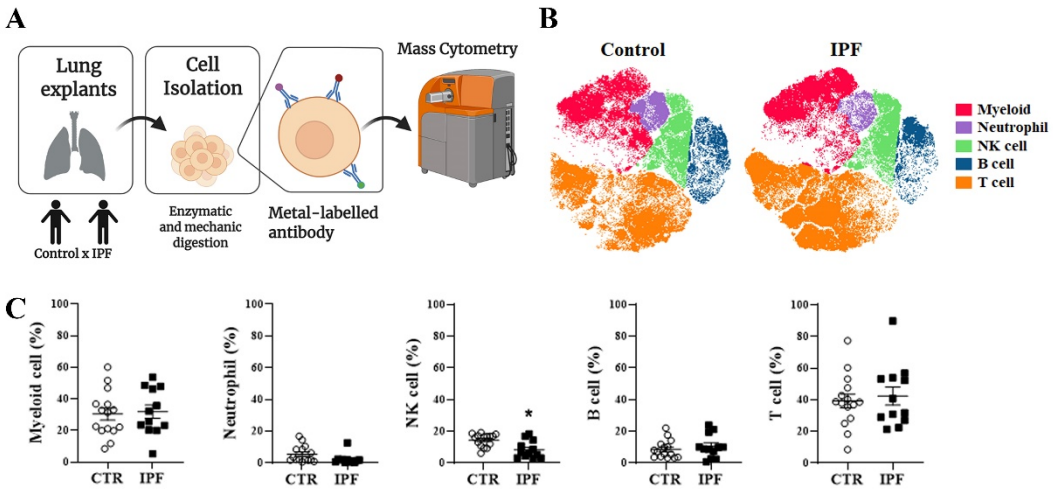

Figure 1: Identification of immune cells in the lungs using high-dimensional single cell maps. (**A**) Representation of cell isolation and antibody labeling for CyTOF analysis. (**B-C**) CyTOF analysis of $CD45^+$ viable ($cisplatin^-$) leukocytes from control (CTR) and IPF lungs. (**B**) viSNE map depicts distribution of color-coded immune cells as indicated. (**C**) Percentage of five different cell types among $CD45^+$ viable leukocytes. Graphical plots represent individual samples showed as mean ± SEM (*=$p<0.05$, Welch's t test, n=15 CTR and n=12 IPF).

Activated memory T cell subsets are over-represented in fibrotic lungs.

We then investigated whether subsets of T lymphocytes were differentially represented in fibrotic lungs. For this analysis, we identified T cells based on expression of CD3 and then subdivided these cells based on CD103 and CD25, two prominent markers that could indicate the presence of tissue resident memory T cell (T_{RM}) and regulatory T cells (T_{REG}) cells. Next, we applied self-organizing map algorithm (FlowSOM) as an unbiased approach to create nodes of phenotypically similar cell types (**Figure 2A, B**). This approach allowed identification of 7 distinct subsets, including: 1) CD4 T effector memory (T_{EM}) ($CD45RO^+$ $CCR7^-$), 2) CD4 T resident memory (T_{RM}) ($CD45RO^+$ $CCR7^-$ $CD103^+$), 3) CD4 T regulatory cell (T_{reg}) ($CD25^+$ $FOXP3^+$ $CCR4^+$ $CD127^-$), 4) CD8 T_{RM} ($CD45RO^+$ $CCR7^-$ $CD103^+$), 5) CD4 T effector memory (T_{EM}) ($CD45RO^+$ $CCR7^-$), 6) CD8 T effector memory re-expressing CD45RA (T_{EMRA}) ($CD45RO^-$ $CCR7^-$), and 7-8) two subpopulations of CD3 T cells with low expression of both CD4 or CD8 [identified as double negative (DN) T cells expressing CD103 (DN T_{RM}) or $CD45RO^+$ CCR7 (DN T_{EMRA})]. These DN T cells have been previously described in lungs and other organs and likely includes NK T cell and γδ T cell subsets. We found that three specific subtypes of memory T cells were over-represented in IPF lungs, including CD4 T_{RM} and CD8 T_{RM} cells and CD8 T_{EMRA} cells (**Figure 2D**).

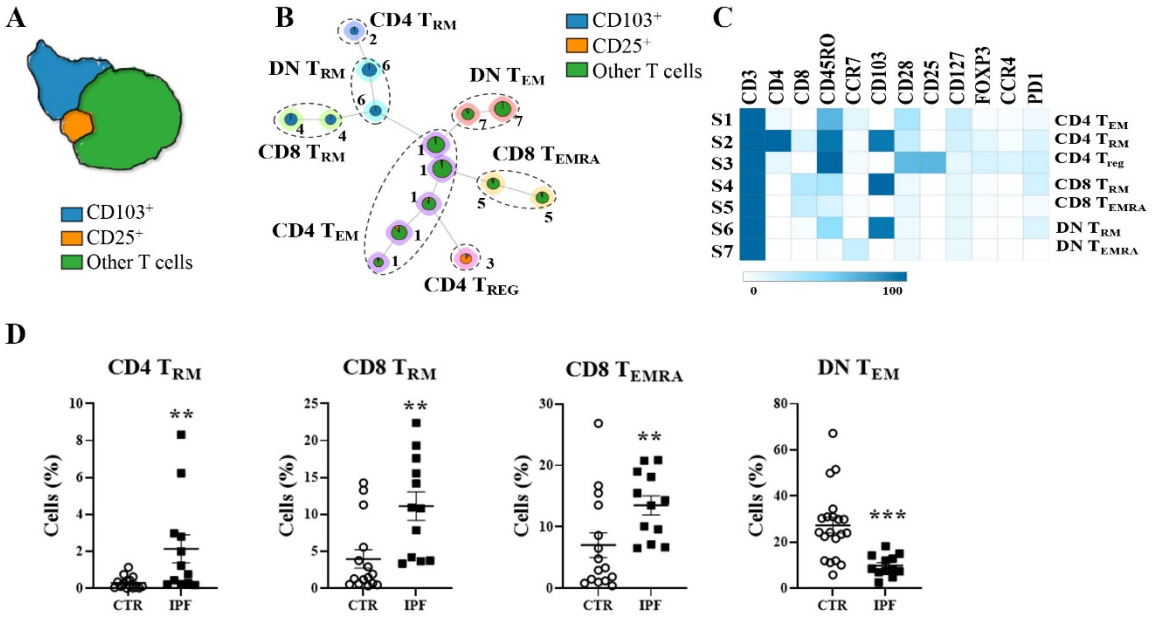

Figure 2: Phenotypic characterization of T cell subsets in IPF lungs. (**A-D**) Mass cytometry analysis of $CD3^+$ T cell gated from $CD45^+$ viable (cisplatin$^-$) leukocytes in the lungs of control or IPF patients. (**A**) viSNE map of T cell were annotated as $CD103^+$ (blue), $CD25^+$ (orange) and other populations of T cells (green). (**B**) Self-organizing map (FlowSOM) shows T cells annotated as CD4 T_{EM}, CD4 T_{RM}, CD4 T_{REG}, CD8 T_{RM}, CD8 T_{EMRA}, and double negative ($CD4^-CD8^-$) DN T_{RM} and DN T_{EMRA} in color-coded pie graphics according to the percentage of CD103 and CD25 expression. (**C**) Heat map shows expression of segregation markers expressed by different subpopulations of T cell (S1-7). (**D**) Percentage of CD4 T_{RM}, CD8 T_{RM}, CD8 T_{EMRA}, and DN T_{EMRA} subpopulations in CTR and IPF lungs. Graphical plots represent individual samples showed as mean ± SEM (*=$p<0.05$, Welch's t test, n=15 CTR and n=12 IPF). (T_{EM}: T effector memory, T_{RM}: T resident memory, T_{REG}: regulatory T cell; T_{EMRA}: effector memory CD45RA).

To further investigate T cell subpopulations over-represented in fibrotic lungs, we analyzed scRNA-seq from a recently reported by our group aimed to characterize populations of epithelial cells and fibroblast. For this new evaluation, T cells (*CD3*$^+$) were extracted, embedded in a uniform manifold approximation and projection map (UMAP), and reclustered. Eight different functionally distinct subpopulations of T cells were identified from UMAP, 3 of which expressed the T_{RM} markers *ITGAE* (CD103) and/or *CD69* (a C-type lectin domain family 2) (**Figure 3A-B**). Subsequent analyses focused on CD4 and CD8 T_{RM} cells since CD8 T_{EMRA} could not be reliably identified in transcriptomic data. CD8 T_{RM} cells expressed *ITGAE*, the transcription factor Hobit *(ZNF683,* zinc finger Protein 683), *GZMB* (granzyme B) and *KLRD1* (CD94) (**Figure 3B**). Two subsets of CD4 T_{RM} were also identified: one that expressed *ITGAE*, high levels of *CD69, ZNF683,* and the Th1-type cytokines *IFNG* and *TNF* [CD4 T_{RM} (*IFNG*$^+$)] and a second subset that expressed lower levels of *ITGAE* and was negative for *IFNG* and *TNF* [CD4 T_{RM} (IFNG$^-$)] (**Figure 3B**). When comparing CD4 T_{RM} (*IFNG*$^+$) and CD4 T_{RM} (*IFNG*$^-$) cells, we observed that CD4 T_{RM} (*IFNG*$^+$) cells expressed 246 genes augmented in disease (IPF versus control lungs), while CD4 T_{RM} (IFNG$^-$) exhibited only 62 augmented genes (**Figure 3C**), further supporting the potential importance of CD4 T_{RM} (*IFNG*$^+$) in IPF.

In addition to CD4 T_{RM} (*IFNG*$^+$) cells, CD8 T_{RM} cells in IPF appeared highly activated with 211 genes whose expression was increased compared to CD8 T_{RM} cells in control lungs (**Figure 3C**). Thus, we performed GO enrichment analysis to identify whether common biological functions could be ascribed to CD4 T_{RM} (*IFNG*$^+$) and CD8 T_{RM} in IPF (WebGestalt). We found that expression of 129 genes was increased in both CD4 T_{RM} (*IFNG*$^+$) and CD8 T_{RM} cells in IPF (**Figure 3D**), with enrichment in the following GO categories: response to interferon-γ, actin filament organization, immune-response signaling pathways, and T cell activation (**Figure 3E**). These data support the conclusion that CD4 and CD8 T_{RM} cells in IPF share a conserved phenotype characterized by IFNγ-driven responses (e.g. - *IFITM2, IFNG, SOCS1, VIM*) and TCR activation (e.g. - *CD2, CD3D, CD6, LAT, LCP1, RAC2)*. These results are part of a manuscript now in revision at the *American Journal of Respiratory Cell and Molecular Biology*.

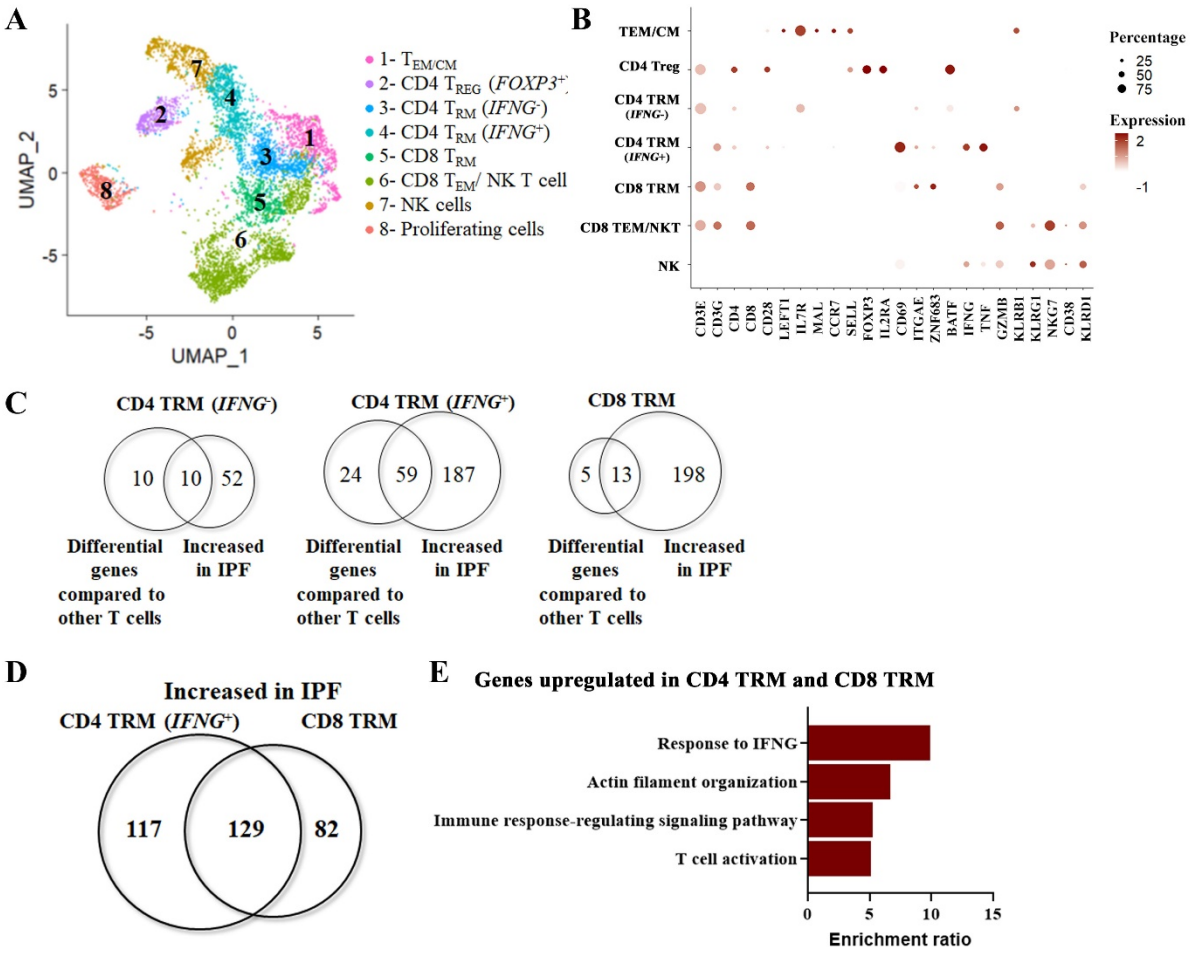

Figure 3: Gene expression and functional characterization of T_{RM} cells in fibrotic lungs. (**A-D**) Single cell RNA-sequencing analysis of resident memory (T_{RM}) in the lungs of control or IPF patients. (**A**) Uniform manifold approximation and projection (UMAP) of lymphocytes from control and IPF lungs shows distribution of CD4 T cell, CD8 T cell, and NK subpopulations as indicated. (**B**) Dot-plot graphic depicting genes expressed by different subsets of lymphocytes. (**C**) Venn-diagram of genes whose expression is increased in CD4 T_{RM} (*IFNG*$^+$) and CD8 T_{RM} cells in IPF lungs compared to control lungs. (**D**) Gene ontology analysis of gene sets enriched in CD4 T_{RM} (*IFNG*$^+$) and CD8 T_{RM} cells from IPF lungs compared to control lungs. Graphic bars represent enrichment ratio with p-value < 0.05.

Identification of a differential T cell response in the blood of CMV$^+$ IPF patients.

To determine the influence of Herpesvirus (primarily CMV) infection on skewing T cell subpopulations during IPF, we isolated total peripheral mononuclear cells from individuals positive or negative for CMV (controls or IPF) and analyzed the cells by CyTOF using the same panel of antibody applied to lung cells. We identified T cells based on expression of CD3 and

then subdivided these cells based on CD4 and CD8 expression (**Figure 3A**). Next, we used FlowSOM as described in above (**Figure 3 B**) and identified 12 distinct subsets of T cells,

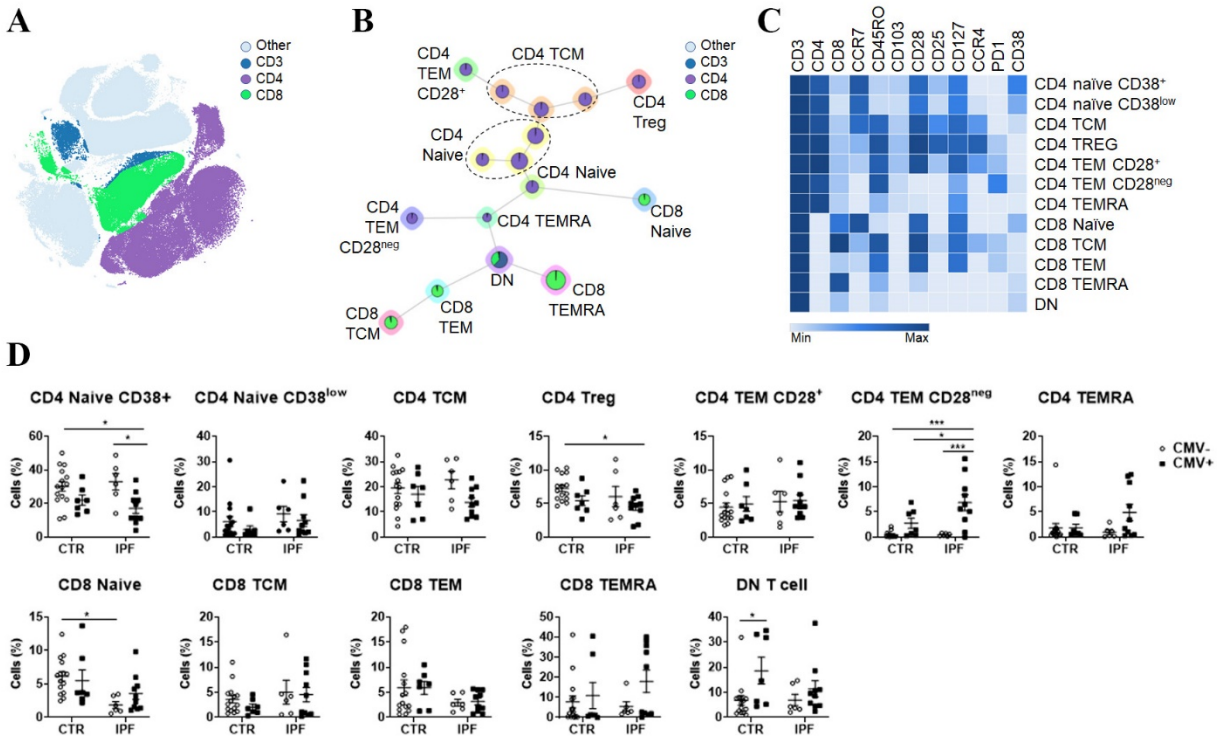

Figure 3: Phenotypic characterization of T cell subsets in peripheral blood of CMV$^+$ and CMV$^-$ IPF patients. (**A-D**) Mass cytometry analysis of CD3$^+$ T cell gated from CD45$^+$ viable (cisplatin$^-$) leukocytes in peripheral blood of control or IPF patients. (**A**) viSNE map of T cell were annotated as CD3$^+$ (dark blue), CD4$^+$ (purple), CD8$^+$ (green) and other populations of leukocytes (light blue). (**B**) Self-organizing map (FlowSOM) shows T cells annotated as CD4 naïve (CD38$^+$ or CD38^{low}), CD4 T_{CM}, CD4 T_{REG} , CD4 T_{EM} (CD28$^+$ and CD28^{neg}), CD4 T_{EMRA}, CD8 naïve, CD8 T_{CM}, CD8 TEM, CD8 T_{EMRA}, and double negative (CD4$^-$CD8^{low}) DN in color-coded pie graphics according to the percentage of CD3, CD4, and CD8 expression. (**C**) Heat map shows expression of segregation markers expressed by different subpopulations of T cells. (**D**) Percentage of T cell subpopulations in CMV$^+$ and CMV$^-$ CTR and IPF patients. Graphical plots represent individual samples showed as mean ± SEM (*=p<0.05, ***<0.0001 two-way ANOVA, n=22 CTR and n=16 IPF). (CTR: control, T_{CM}: T central memory, T_{EM}: T effector memory, T_{REG}: regulatory T cell; T_{EMRA}: effector memory CD45RA).

including: CD4 and CD8 naïve (CD45RO$^-$ CCR7$^+$), CD4 and CD8 T central memory (CD45RO$^+$ CCR7$^+$), CD4 and CD8 T effector memory (T_{EM}) (CD45RO$^+$ CCR7$^-$), CD4 T regulatory cell (T_{reg}) (CD25$^+$ CCR4$^+$), CD4 and CD8 T effector memory re-expressing CD45RA (T_{EMRA}) (CD45RO$^-$ CCR7$^-$), and one subpopulations of CD3 T cells with low expression of both CD4 or CD8 [identified as double negative (DN) CD45RO$^-$ CCR7- (DN T_{EMRA})]. Further, two populations of CD4 naïve and two populations of CD4 T_{EM} cells were observed (CD4 naïve CD38$^+$ and CD38^{low} and CD4 T_{EM} CD28$^+$ and CD28^{negative}) (**Figure 3C**). We then found that CD4 naïve T cells (CD38$^+$) were significantly decreased in CMV$^+$ IPF patients when compared to CMV$^-$ controls and CMV$^-$ IPF patients. CD4 T_{reg} were also decreased in CMV$^+$ IPF patients when compare to CMV$^-$ controls. Interestingly, CD4 T_{EM} CD28^{negative} cells were increased in CMV$^+$ IPF subjects when compare to all other groups (**Figure 3D**). These results indicate that these CD4 T cells could represent an important biomarker in this disease. While T lymphocytes expressing low levels of CD28 has been reported in IPF and associated with accelerated disease progression, we found that increased CD28$^-$ T cells in IPF could be driven by the presence of

CMV. Whether these cells are related to the CD4 T_{RM} cells over-represented in the lungs of IPF patients is still unclear. Mouse model studies outlined below should help us to understand the origin and functions of CD4 TEM/TRM cells in IPF.

Aim 2:

Major task 1: Determine whether alveolar epithelial endoplasmic reticulum stress affects quantitative or functional T cell responses to herpesvirus infection.

We obtained a tamoxifen-inducible transgenic mouse line carrying a cysteine-to-glycine substitution at codon 185 of the surfactant protein gene (*SftpcC185G*) from Dr. Michael Beers, Univ. of Pennsylvania. *SftpcC185G*-expressing mice were shown to generate a gain-of-function mutant protein that results in misfolding and ER stress in type 2 AECs, following induction with tamoxifen. Our first approach was to test the extension of lung fibrosis *SftpcC185G* mice develop following tamoxifen challenge. Homozygous *SftpcC185G* mice were treated with 200mg/kg of tamoxifen/day via intraperitoneal injection twice as indicated in **Figure 4A**. Mice weight was measured for 14 days as an indication of disease severity. We observed that 46% (7 out of 15) of *SftpcC185G* mice showed progressive weight lost (≥20% at day 11) (**Figure 4B**). At day 14, mice were euthanized and $CD45^-$ cells (non-leukocytes) were isolated for subsequent investigation of spliced and unspliced *Xbp1* mRNA expression. A RT-PCR reaction showed that mice with ≥20% of weight lost exhibited increase in *sXbp1/uXbp1* ratio, signling endoplasmic reticulum stress in mice with more substancial weight lost following tamoxifen (**Figure 4C**).

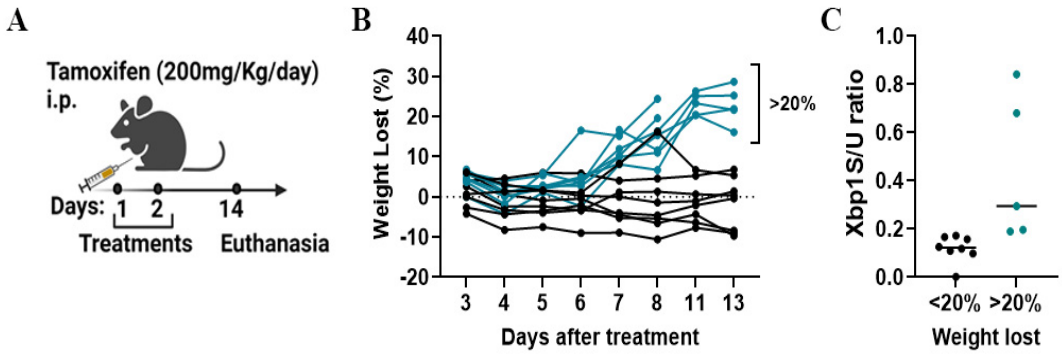

Figure 4: Tamoxifen-treated *Stpc*C185G/Rs26Cre mice exhibit weight lot and increased ER stress marker. (**A**) Schematic representation of tamoxifen treatment protocol used for *Stpc*C185G/Rs26Cre mice. (**B**) percentage of weight lost measured in *Stpc*C185G/Rs26Cre mice after different days after tamoxifen injections. (**C**) Ratio expression of sXb1/uXbp1 in CD45- cells isolated from *Stpc*C185G/Rs26Cre mice at day 14 after tamoxifen. Graphs are mean ± SEM, n=15 mice.

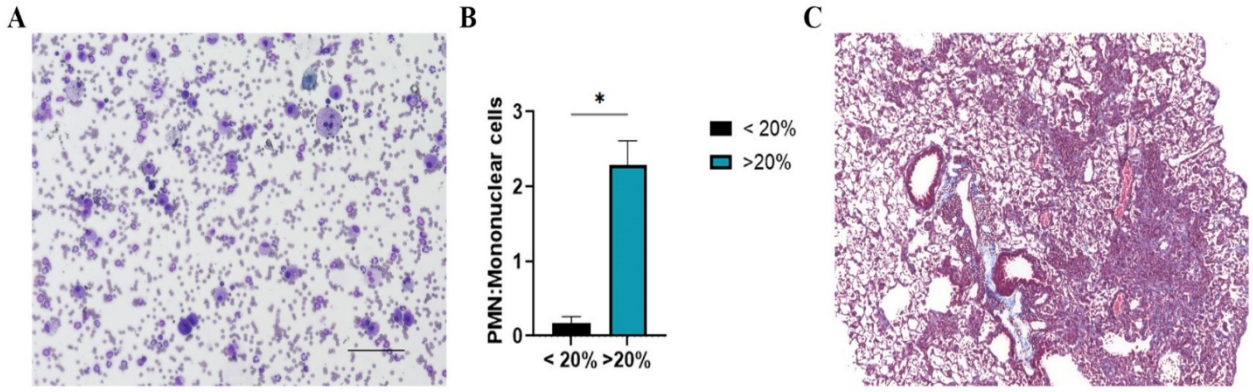

Figure 5: *Stpc*C185G/Rs26Cre mice exhibit inflammation and fibrosis. (**A**) Cytospin of BAL cells (day 14 after tamoxifen) from a representative *Stpc*C185G/Rs26Cre mice that showed ≥20% of weight lost. (**B**) Ratio of PMN by mononuclear cells counts observed in BAL. (C) Lung section stained with trichrome of StpcC185G/Rs26Cre mice at day 14 of tamoxifen treatment. Graphs are mean ± SEM, n=15 mice. Magnification 10x.

Next, we analyzed the BAL from these mice and observed a prominent infiltration of PMN cells in mice with a weight lost ≥20% **Figure 5A and B**. We also observed development of fibrotic remodeling by trichrome staining of lung sections. Thus, we demonstrated that *SftpcC185G* mice can be used as a model of alveolar epithelial ER stress and pulmonary fibrosis. We are now preparing experiments where mice will be challenged with murine herpesvirus (MHV68) and later injected with tamoxifen. We expect that these two components would potentiate ER stress in alveolar epithelial cells, and result in a more extensive fibrotic response. We plan to use this model to quantify T cell subsets after herpesvirus infection and perform T-cell functional studies, including co-culture studies with epithelial cells.

Major task 2: Determine whether antiviral treatment can prevent or reverse T-cell exhaustion.
These studies have not been performed yet but will be completed during the next year.

Major task 3: Test whether therapies designed to prevent or reverse T-cell exhaustion can enhance viral clearance and prevent lung fibrosis.
These studies have not been performed yet but will be completed during the next year.

- **What opportunities for training and professional development has the project provided**
  Dr. Serezani, an early-career investigator, has presented this work at a major international conference, and developed advanced computational skills analyzing mass-cytometry data.

- **How were the results disseminated to communities of interest?**
  Abstract presented at the annual American Thoracic Society meeting: Serezani A, Pascoalino B, Vowell K, Kropski J, Blackwell TS. T Cell Phenotype in Peripheral Blood of Patients with Idiopathic Pulmonary Fibrosis. Proc. Am. Thorac. Soc. 199:A2425, 2019.
  Manuscript: Ana PM Serezani, Bruno D. Pascoalino, Julia Bassano, Katherine Joy Nicholas Vowell, Erin M Wilfong, Peggy L Kendal, Jonathan A Kropski, Timothy S Blackwell. Inflammatory landscape in Idiopathic Pulmonary Fibrosis. American Journal of Respiratory Cell and Molecular Biology, in revision.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  During the final year, we will continue studies as outlined in the SOW. In each section above, we have

outlined our specific plans for finishing the proposed work.

For aim 2, we had to overcome a substantial hurdle to obtain and characterize a new mouse model after developing issues with the mice originally proposed for these studies. However, we now have a functional model in hand and will perform animal studies as outlined in the SOW.

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  Nothing to Report.
- **What was the impact on other disciplines?**
  Nothing to Report.
- **What was the impact on technology transfer?**
  Nothing to Report.
- **What was the impact on society beyond science and technology?**
  Nothing to Report.

5. CHANGES/PROBLEMS:

As described in last year's report, we had issues with our animal model and have made the necessary changes in order to complete the proposed studies. We obtained IACUC approval for this change in mouse models.

6. PRODUCTS:

Publications, conference papers, and presentations:

- **Journal publications.**
- Manuscript: Ana PM Serezani, Bruno D. Pascoalino, Julia Bassano, Katherine Joy Nicholas Vowell, Erin M Wilfong, Peggy L Kendal, Jonathan A Kropski, Timothy S Blackwell. Inflammatory landscape in Idiopathic Pulmonary Fibrosis. American Journal of Respiratory Cell and Molecular Biology, in revision.

- **Books or other non-periodical, one-time publications.**
  Nothing to Report.
- **Other publications, conference papers, and presentations.**
  Serezani A, Pascoalino B, Vowell K, Kropski J, Blackwell TS. T Cell Phenotype in Peripheral Blood of Patients with Idiopathic Pulmonary Fibrosis. Proc. Am. Thorac. Soc. 199:A2425, 2019.
- **Website(s) or other Internet site(s)**
  Nothing to Report.
- **Technologies or techniques**
  Nothing to Report.
- **Inventions, patent applications, and/or licenses**
  Nothing to Report.
- **Other Products**
  Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name: Timothy S. Blackwell, M.D.

Project Role: P.I.
Researcher Identifier (e.g. ORCID ID): 0000-0002-0337-7052
Nearest person month worked: 1
Contribution to project: Dr. Blackwell manages the project. Dr. Blackwell oversees studies measuring the immune response to herpesviruses and he oversee all animal experiments. He participates in data interpretation, presentation, and publication.
Funding support: N/A

Name: Peter Ghattas
Project Role: Research Assistant II
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 3
Contribution to project: Mr. Ghattas assists with Dr. Serezani with performing immunology studies and is now responsible for processing and storing human and mouse samples.
Funding support: N/A

Name: Susan Martin
Project Role: LPN Research
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 4
Contribution to project: Ms. Martin serves as the study coordinator for this project. She is responsible for enrollment, organization of study visits, and data collection.
Funding support: N/A

Name: Ana Serezani
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0002-7191-9730
Nearest person month worked: 0.6
Contribution to project: Dr. Serezani is responsible for performing and interpreting the human and animal immunology studies, including flow cytometry, mass cytometry, and T-cell functional studies.
Funding support: N/A

Name: Taylor Sherrill
Project Role: Lab Manager
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.95
Contribution to project: Mr. Sherrill is responsible for the mouse colony for this project. He also performs and evaluates mouse model studies.
Funding support: N/A

Name: Fabian Maldonado
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 0.3
Contribution to project: Dr. Maldonado performs and oversees study-related bronchoscopy procedures and sample acquisition.
Funding support: N/A

Name: Vasiliy Polosukhin
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0001-9845-8041
Nearest person month worked: 1.2
Contribution to project: Dr. Polosukhin is responsible for immunohistochemical studies staining for herpesviruses in lung biopsy samples. He is also responsible for histopathological endpoints in animal experiments.
Funding support: N/A

Name: Carleen Sabusap
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0002-5039-8825
Nearest person month worked: 1.75
Contribution to project: Dr. Sabusap is responsible for assisting Dr. Blackwell with study design, working with the clinical coordinator and the Redcap database, and project oversight related to the project aims.
Funding support: N/A

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Other Support Changes:

Timothy S. Blackwell, M.D.
New: VUMC85126 (W81XWH2010540) (Blackwell)
Ended: 5 R01 HL131906-04

Fabien Maldonado, M.D.
New: VUMC88362 (U01CA253166) (Maldonado); VUMC85862 (Maldonado); 1 R01 CA253923-01A1 (Massion)
Ended: None.

Vasiliy V. Polosukhin, M.D., Ph.D., Sc.D.
New: 1 R01 HL157373-01 (Benjamin); 1 R01 HL157583-01 (Dikalov); 2 R01 GL122554-07A1 (Newcomb)
Ended: N/A

Ana Serezani, Ph.D.
New: N/A
Ended: N/A

- **What other organizations were involved as partners?**

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

- COLLABORATIVE AWARDS: N/A
- QUAD CHARTS: N/A

9. APPENDICES: N/A