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14. ABSTRACT

Purpose: To test the hypothesis that the replacement of tissue-resident alveolar macrophages with monocyte-derived macrophages in response to repeated injury over the lifespan explains the delayed onset of lung fibrosis until the later decades of life. We will use our experimental findings in mice to guide an unbiased assessment of macrophage heterogeneity in normal human lungs and lungs from patients with pulmonary fibrosis. The overarching goal of these studies is to identify clinically applicable biomarkers that can guide therapy and factors released from tissue-resident macrophages or bone marrow-derived macrophages that prevent or promote fibrosis, respectively so they can be targeted for prevention or therapy.

Aim 1: To determine whether replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages during aging contributes to the enhanced susceptibility to lung fibrosis in aged mice. Mice are currently being aged. The work is continuing according to SOW, no major findings to report. However, we have generated improved, more economical and robust mouse model to distinguish tissue-resident and monocyte-derived alveolar macrophages.

Aim 2: To determine whether tissue-resident alveolar macrophages or monocyte-derived alveolar macrophages differentially respond to epithelial injury in a murine model of accelerated pulmonary fibrosis. The work on this aim is in progress, in accordance with SOW, no major findings to report at the moment (expected in the year 2 of award).

Aim 3: To identify novel biomarkers expressed by human tissue-resident and monocyte-derived alveolar macrophages based on single cell molecular classification in patients with pulmonary fibrosis. We have recruited a planned number of patients into the study and performed transcriptional profiling by means of RNA-seq on single cell and bulk sorted populations or alveolar macrophages. Major findings: In agreement with our hypothesis, using single cell RNA-seq, we found substantial heterogeneity of alveolar macrophages in the healthy donor lung, we now sequencing and analyzing data from patients with lung fibrosis. In addition, we found that gene expression signature of profibrotic monocyte-derived alveolar macrophages generated in mouse models of lung fibrosis was present in the alveolar macrophages of patients with various forms of lung fibrosis.

15. SUBJECT TERMS
alveolar macrophages, pulmonary fibrosis

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Introduction

Purpose: To test the hypothesis that the replacement of tissue-resident alveolar macrophages with monocyte-derived macrophages in response to repeated injury over the lifespan explains the delayed onset of lung fibrosis until the later decades of life. We will use our experimental findings in mice to guide an unbiased assessment of macrophage heterogeneity in normal human lungs and lungs from patients with pulmonary fibrosis. The overarching goal of these studies is to identify clinically applicable biomarkers that can guide therapy and factors released from tissue-resident macrophages or bone marrow-derived macrophages that prevent or promote fibrosis, respectively so they can be targeted for prevention or therapy. This hypothesis will be tested in the following aims: Aim 1: To determine whether replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages during aging contributes to the enhanced susceptibility to lung fibrosis in aged mice. Aim 2: To determine whether tissue-resident alveolar macrophages or monocyte-derived alveolar macrophages differentially respond to epithelial injury in a murine model of accelerated pulmonary fibrosis. Aim 3: To identify novel biomarkers expressed by human tissue-resident and monocyte-derived alveolar macrophages based on single cell molecular classification in patients with pulmonary fibrosis.

Keywords

Alveolar macrophages, pulmonary fibrosis, idiopathic pulmonary fibrosis

Accomplishments

Major Goal: Specific Aim 1: To determine whether replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages during aging contributes to the enhanced susceptibility to lung fibrosis in aged mice.

Major Task 1: Are tissue-resident alveolar macrophages replaced by monocyte-derived alveolar macrophages during normal aging?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will monitor animals, Dr. Budinger will oversee the project.
Accomplishments: Generation of the shielded bone marrow chimeras is complete (100%), mice are currently being aged as planned to be analyzed during the second year of the project.
Other achievements: As an indirect result of this project, we have developed a novel, more robust and economic, fate mapping system based on CD11c-ER-Cre and CX3CR1-ER-Cre mice crossed to fluorescent reporter (see figure 1). This system should be able to replace shielded bone marrow chimeras in the future studies and increase throughput, accuracy and cost-effectiveness of our research. We are currently testing these reporter mice to validate our findings from shielded chimeras.

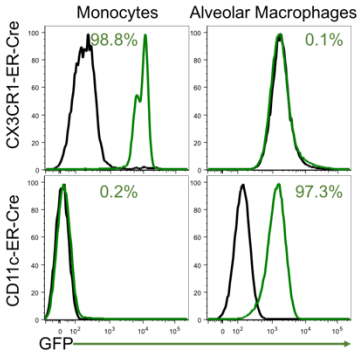

Figure 1: Strategies for efficient targeting of Tissue-Resident and Monocyte-Derived Alveolar Macrophages. CX3CR1-ER-Cre and CD11c-ER-Cre mice were crossed to zsGreen (GFP) reporter mice and offsprings were exposed to tamoxifen chow for 1 and 7 weeks, correspondingly. Alveolar macrophages and monocytes were identified using our previously published gating strategy (Misharin et al., AJRCMB, 2013). CX3CR1-ER-Cre efficiently labeled 100% of circulating monocytes, without affecting tissue-resident alveolar macrophages, which do not express *Cx3cr1*. CD11c-ER-Cre mice labeled nearly 100% of tissue-resident alveolar macrophages (which express high levels of CD11c), without labeling any circulating monocytes (which do not express CD11c).

Subtask 2: Aging of shielded bone marrow chimeric mice. Monitor shielded bone marrow chimeric mice during aging. Track mortality (Drs. Misharin, Soberanes, Chen). Harvest lungs from shielded chimeric at designated time points (6 weeks, 6, 12, 18 and 24 months), FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Soberanes, Bartom and Chen).
Accomplishments: Generation of the shielded bone marrow chimeras is complete (100%), and 6 weeks and 6 months time points have already been harvested and FACSorted. Animals for 12, 18 and 24 months are being aged. No technical difficulties were encountered.

Major Task 2: Do insults associated with normal aging accelerate the replacement of tissue-resident alveolar macrophages with monocyte-derived alveolar macrophages?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice. Dr. Misharin will perform the procedure, Drs. Soberanes and Chen will assist and monitor animals, Dr. Budinger will oversee the project.
Accomplishments: Generation of the shielded bone marrow chimeras is complete (100%), mice are currently being aged as planned to be analyzed during the second year of the project.

Subtask 2: Infect shielded chimeric mice with three strains of influenza A (A/WSN/33 and A/PR8/34 and A/Udorn/72) at 2, 4 and 6 months of age. Dr. Soberanes will perform the infections, Drs. Soberanes and Chen will assist and monitor animals. Dr. Budinger will oversee the project. Harvest lungs from shielded chimeric at designated time points (6 weeks, 6, 12, 18 and 24 months), FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Bartom, Soberanes and Chen).
Accomplishments: Shielded bone marrow chimeras were infected with influenza A virus (WSN strain). Six weeks and 6 months time points have already been harvested and FACSorted. Animals for 12, 18 and 24 months are being aged. We are awaiting for preliminary results from infection with WSN virus prior to initiating studies with PR8 or Udorn virus.

Major Task 3: Does replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages lead to exaggerated fibrosis in mice?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice on using CD11c-DTR mice as a host. Dr. Misharin will perform the procedure, Drs. Soberanes and Chen will assist and monitor animals, Dr. Budinger will oversee the project.
Subtask 2: Initiate bleomycin-induced lung fibrosis in shielded chimeric mice and harvest macrophage populations. Dr. Soberanes will instill mice with bleomycin, Drs. Chen and Soberanes will assist and monitor animals. Dr. Budinger will oversee the project. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Bartom, Soberanes and Chen).
Accomplishments: During the initial phase experiments, we found out that CD11c-DTR mice develop severe lung injury upon depletion of CD11c-DTR-positive alveolar macrophages. These findings were recently confirmed and reported by another group (Mann et al., Eur J Immunol, 2016). To overcome this issue, we have developed an alternative system for depletion of tissue-resident alveolar macrophages using intratracheal instillation of clodronate-loaded liposomes. We found that intratracheal instillation of 50 ul of clodronate-loaded liposomes efficiently depletes tissue-resident alveolar macrophages and leads to the repopulation of the niche by monocyte-derived alveolar macrophages. Thus, we were able to overcome the technical issues and use regular shielded bone marrow chimeras for this subtask. We have generated sufficient number of shielded bone marrow chimeras and treated them with clodronate-loaded liposomes to deplete tissue-resident alveolar macrophages and induce repopulation with monocyte-derived alveolar macrophages.

Major Task 4: Can adoptive transfer of tissue-resident or monocyte-derived alveolar macrophages rescue or exacerbate, respectively, the severity of experimental lung fibrosis?
Subtask 1: Sort alveolar macrophages, generate bone marrow derived macrophages, perform adoptive transfer and induce lung fibrosis. Dr. Misharin will perform sorting for alveolar macrophages and generate bone marrow derived macrophages in vitro. Dr. Soberanes will perform adoptive transfer of alveolar and bone marrow-derived macrophages and instill mice with bleomycin. Drs. Chen and Soberanes will assist and monitor animals. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Bartom, Soberanes and Chen).

Accomplishments: We have initiated work on this subtask and have optimized the adoptive transfer technique for both tissue-resident and monocyte-derived alveolar macrophages. The experiments are currently ongoing. No preliminary data to report at the moment.

Specific Aim 2: To determine whether tissue-resident alveolar macrophages or monocyte-derived alveolar macrophages differentially respond to epithelial injury in a murine model of the accelerated pulmonary fibrosis.

Major Task 1: Do macrophages from young and aged mice differentially regulate chronic stress in the lung epithelium?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice with tissue-resident or monocyte-derived alveolar macrophages using young and aged CD11c-DTR mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will monitor animals, Dr. Budinger will oversee the project.
Subtask 2: Sort tissue-resident alveolar macrophages, perform adoptive transfer and induce lung fibrosis. Dr. Misharin will perform sorting for alveolar macrophages. Dr. Soberanes will perform adoptive transfer of alveolar and instill mice with bleomycin. Ms. Saber and Dr. Soberanes will assist and monitor animals. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Bartom, Soberanes and Chen).
Subtask 3: Bleomycin-induced lung fibrosis in bone marrow chimeric mice. Monitor shielded bone marrow chimeric mice during aging. Track mortality (Drs. Misharin, Soberanes, Chen). Harvest lungs from shielded chimeric at designated time points, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Soberanes and Chen).
Accomplishments: See above, Aim 1, Major task 4. During the initial phase experiments, we found out that CD11c-DTR mice develop severe lung injury upon depletion of CD11c-DTR-positive alveolar macrophages. These findings were recently confirmed and reported by another group (Mann et al., Eur J Immunol, 2016). To overcome this issue, we have developed an alternative system for depletion of tissue-resident alveolar macrophages using intratracheal instillation of clodronate-loaded liposomes. We found that intratracheal instillation of 50 ul of clodronate-loaded liposomes efficiently depletes tissue-resident alveolar macrophages and leads to the repopulation of the niche by monocyte-derived alveolar macrophages. Thus, we were able to overcome the technical issues and use regular shielded bone marrow chimeras for this subtask. We have generated sufficient number of shielded bone marrow chimeras and treated them with clodronate-loaded liposomes to deplete tissue-resident alveolar macrophages and induce repopulation with monocyte-derived alveolar macrophages.

Major Task 2: Does chronic stress in the epithelium accelerate the replacement of tissue-resident alveolar macrophages with monocyte-derived alveolar macrophages during aging?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice using HPS-/- mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will assist and monitor animals, Dr. Budinger will oversee the project.
Accomplishments: See above, Major Task 2. While HPS-/- mice provided a valuable insight during the preliminary work, their utility was undermined because HPS deficiency was global and affecting not only epithelial cells, but all other cell types as well (including alveolar macrophages).

Fortunately, soon after the work on the grant was initiated, a new strain of mice specifically causing stress in the lung epithelium by targeting telomerase (*Trf1*) in alveolar type 2 epithelial cells using highly specific *Cre*^{Sftpc} driver became available (Povedano et al., 2015, Cell Rep). These mice develop spontaneous age-dependent lung fibrosis. We have initiated collaboration with Dr. Mary Blasco and obtained *Trf1*-floxed mice for our colony. We have now finished crossing these mice to *Cre*^{Sftpc} mice and initiated the proposed experiments. While this change of tools delayed the work on this subtask, it did not change the scope of our work. Moreover, this approach is much more specific and will allow us to reduce the number of required animals.
Subtask 2: Harvest and analyze the lungs. Harvest lungs from shielded chimeric at designated time points (6 weeks, 4, 12 and 18 months), FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Bartom, Soberanes and Chen).
Accomplishments: The work on this subtask was delayed because of the change in the mouse strain (see above). Once the experiments in Subtask 1 are complete, we do not anticipate any further delays in completing Subtask 2 as we have all protocols established and optimized.

Major Task 3: Can the adoptive transfer of tissue-resident alveolar macrophages improve chronic stress in the lung epithelium?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice using HPS-/- mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will assist and monitor animals, Dr. Budinger will oversee the project.
Accomplishments: See above, Major Task 2. While HPS-/- mice provided a valuable insight during the preliminary work, their utility was undermined because HPS deficiency was global and affecting not only epithelial cells, but all other cell types as well (including alveolar macrophages). Fortunately, soon after the work on the grant was initiated, a new strain of mice specifically causing stress in the lung epithelium by targeting telomerase (*Trf1*) in alveolar type 2 epithelial cells using highly specific *Cre*^{Sftpc} driver became available (Povedano et al., 2015, Cell Rep). These mice develop spontaneous age-dependent lung fibrosis. We have initiated collaboration with Dr. Mary Blasco and obtained *Trf1*-floxed mice for our colony. We have now finished crossing these mice to *Cre*^{Sftpc} mice and initiated the proposed experiments. While this change of tools delayed the work on this subtask, it did not change the scope of our work. Moreover, this approach is much more specific and will allow us to reduce the number of required animals.
Subtask 2: Sort alveolar macrophages, perform adoptive transfer and induce lung fibrosis. Dr. Misharin will perform sorting for alveolar macrophages. Dr. Soberanes will perform adoptive transfer of alveolar and instill mice with bleomycin. Ms. Saber and Dr. Soberanes will assist and monitor animals. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Soberanes and Chen).
Accomplishments: The work on this subtask was delayed because of the change in the mouse strain (see above). Once the experiments in Subtask 1 are complete, we do not anticipate any further delays in completing Subtask 2 as we have all protocols established and optimized.

Specific Aim 3: To identify novel biomarkers expressed by human tissue-resident and monocyte-derived alveolar macrophages based on single cell molecular classification in patients with IPF.
Major Task 1: Collect samples, perform RNA-seq on populations of lung macrophages, perform single cell RNA-seq and analyze the data.

Subtask 1: Submit documents for local IRB review. Organize workflow, establish standard operating procedures, meeting with all co-investigators.

Accomplishments: Our study protocol was approved by the local IRB on 2016/06/05. This protocol was reviewed by the US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable DOD, US Army, and USAMRMC human subjects protection requirements. We received an initial note of approval on 2015/12/01 and HRPO Approval Memorandum on 2016/1/9, HRPO Log Number A-18899.b.

We have completed all optimization studies and established standard operation procedures. We have **published** the results of these preliminary studies (Bharat et al., 2016, AJRCMB) and **presented** these data at the American Thoracic Society meeting (San Francisco, CA) in the the poster format. Our publication was accompanied by the editorial (Ballinger and Christman, 2016, AJRCMB) and at the time of this report listed among highly accessed on AJRCMB website. Importantly, our protocol and findings were independently validated by two groups (Yu at al., 2016, AJRCMB; Desch et al., 2016, AJRCCM).

During 2015-2016 we have conducted monthly meetings between all collaborators (Drs. Mutlu, Bhorade, Budinger) to align the work on the project on both study sites and coordinate efforts.

Subtask 2: Begin study. Recruit first patients to the study (Drs. Mutlu, Bhorade, Budinger). Perform pilot RNA-seq on populations of pulmonary macrophages, establish optimal workflow and data analysis pipelines (Drs. Misharin, Budinger, Bartom, Berdnikovs, Perlman).

Accomplishments: We have achieved a significant progress on this subtask. Since approval of the protocol by IRB and HRPO a total of 45 lung samples were processed, including donor lungs and lungs from patients with various forms of lung fibrosis (SSc-ILD, IPF, ILD, MCTD, hypersensitivity pneumonitis, pneumoconiosis, CPFE). As proposed, we have FACSorted alveolar macrophages and alveolar type 2 cells from these samples and extracted RNA for gene expression profiling using RNA-seq. In addition, to emphasize the advantages of the focusing on specific cellular populations we analyzed gene expression profiles from the whole lung tissue (traditional approach implemented by other groups). All samples have yielded high-quality (RIN over 7) RNA. Libraries for RNA-seq were prepared using a high-throughput automated robotic platform (Agilent Bravo) to minimize a batch effect, all libraries have passed the QC. Libraries from were sequenced on Illumina NextSeq 500 instrument. Data was processed using an established bioinformatic pipeline on high-performance computational cluster QUEST (Dr. Bartom). Principal component analysis revealed a strikingly high degree of structure in alveolar macrophage and alveolar type 2 cell datasets, as opposed to whole lung tissue, which revealed very little structure (see Figure 2). While the analysis is still ongoing, the preliminary analysis revealed a substantial amount of heterogeneity among the patients with lung fibrosis.

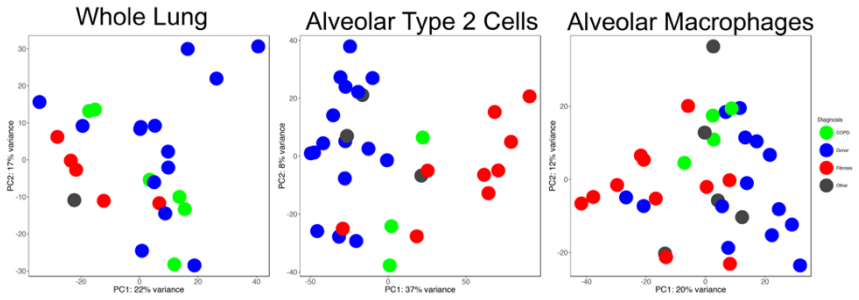

Figure 2: Principal component analysis of the gene expression profiles for the whole lung and FACSorted alveolar type 2 cells and alveolar macrophages. Blue dots indicate donors, red dots – pulmonary fibrosis, green dots – COPD, grey dots – ILD.

Subtask 3: Single cell RNA-seq. Recruit patients to the study (Drs. Mutlu, Bhorade, Budinger). Process samples, generate single-cell RNA-seq libraries (Drs. Misharin, Chen, Soberanes). Perform RNA-seq, perform data analysis (Drs. Misharin, Budinger, Bartom, Berdnikovs, Perlman).

Accomplishments: We have established collaboration with Dr. Ido Amit, Weizmann Institute of Science, Israel, one of the world's leading experts in single cell RNA-seq. Using the materials provided by Amit lab, we have performed single-cell sorting on a subset of donor and fibrotic lungs and, subsequently, performed single-cell RNA-seq using MARS-seq protocol on two donor lungs (Figure 3). Preliminary analysis of these data 1) confirmed the flow cytometry markers that we have previously identified for bulk population sorting (see Subtask 2) and 2) revealed heterogeneity within the population of alveolar macrophages. Currently, we are working on establishing Amit's lab MARS-seq protocol in our laboratory. This will allow us to utilize our own sequencing resources, rather than using sequencing facility at University of Chicago and, as a result, have better control over the sequencing and, potentially, reduce the cost.

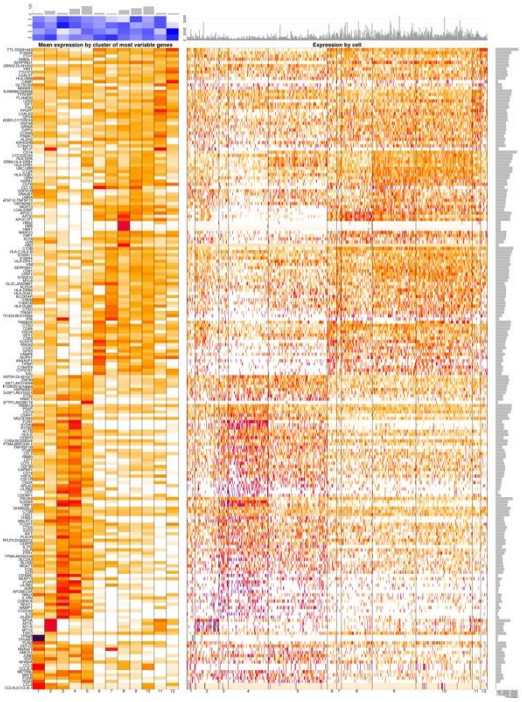

Figure 3: Single-cell RNA-seq reveals heterogeneity of alveolar macrophages in the healthy donor lung. Single cell sorting was performed on FACS Aria III instrument using our recently published protocol (Bharat et al., AJRCMB, 2016) using "index sort" mode. After gating on $CD45^+$ live, single cells, neutrophils were excluded as $CD15^+$ cells and all cells in HLA-DR^+ gate (which included monocytes, interstitial macrophages, alveolar macrophages, dendritic cells and some B cells) were sorted into 384-well plates containing capture buffer. Plates were snap frozen and stored until processing. Single cell RNA-seq was performed using MARS-seq protocol (Jaitin et al., Science, 2014). Overall sorting efficiency was 80%. Right panel: each column corresponds to an individual cell, each raw corresponds to an individual gene, both hierarchical clustered to reveal the clusters of cells with similar gene expression profiles. Left panel is a summary of the findings from the right panel: cells were grouped into 12 clusters, guide genes associated with each cluster are shown on the left. Alveolar macrophages correspond to the most abundant clusters 8, 9 and 10.

Major Task 2: Design diagnostic flow cytometry panel.

Subtask 1: Select prospective markers based on RNA-seq data (Drs. Misharin, Budinger, Berdnikovs, Perlman). Screen antibodies for panel design (Dr. Misharin). Design and validate the panel (Drs. Misharin, Budinger).

Accomplishments: Since this task is interdependent on the results from Major Task 1, currently, we have nothing to report.

Opportunities for training and professional development:

On March 30th, 2016 Dr. Misharin participated as a panel member at the webinar "Flow Cytometric Analysis of Myeloid Cells in Human Blood, Bronchoalveolar Lavage, and Lung Tissues" organized by American Thoracic Society. He presented findings from our group (Aim 3, Task 2; Bharat et al., AJRCMB, 2016).

Impact on society beyond science and technology: Nothing to report.

Impact

Impact on the development of the principal discipline(s) of the project: Nothing to report.

Impact on other disciplines: Nothing to report.

Impact on technology transfer: Nothing to report.

Impact on society beyond science and technology: Nothing to report.

Changes/Problems

Changes in approach:

Aim 1, Major task 4. During the initial phase experiments, we found out that CD11c-DTR mice develop severe lung injury upon depletion of CD11c-DTR-positive alveolar macrophages. These findings were recently confirmed and reported by another group (Mann et al., Eur J Immunol, 2016). To overcome this issue, we have developed an alternative system for depletion of tissue-resident alveolar macrophages using intratracheal instillation of clodronate-loaded liposomes. We found that intratracheal instillation of 50 ul of clodronate-loaded liposomes safely and efficiently depletes tissue-resident alveolar macrophages and leads to the repopulation of the niche by monocyte-derived alveolar macrophages. Thus, we were able to overcome the technical issues and use regular shielded bone marrow chimeras for this subtask. We have generated sufficient number of shielded bone marrow chimeras and treated them with clodronate-loaded liposomes to deplete tissue-resident alveolar macrophages and induce repopulation with monocyte-derived alveolar macrophages.

Aim 2, Major Task 2. Initially, we proposed to use HPS-/- mice and a series of adoptive transfer experiments to dissect relationship between stress in alveolar epithelial cells, alveolar macrophages and susceptibility to lung fibrosis. While HPS-/- mice provided a valuable insight during the preliminary work, their utility was undermined because HPS deficiency was global and affecting not only epithelial cells, but all other cell types as well (including alveolar macrophages). Fortunately, soon after the work on the grant was initiated, a new strain of mice specifically causing stress in the lung epithelium by targeting telomerase (*Trf1*) in alveolar type 2 epithelial cells using highly specific Cre^{Sftpc} driver became available (Povedano et al., 2015, Cell Rep), these mice develop spontaneous age-dependent lung fibrosis. We have initiated collaboration with Dr. Mary Blasco and obtained *Trf1*-floxed mice for our colony. We have now finished crossing these mice to Cre^{Sftpc} mice and initiated the proposed experiments. While this change of tools delayed the work on this task, it did not change the scope of our work. Moreover, this approach is much more specific and will allow us to reduce the number of required animals.

Actual or anticipated problems or delays and actions or plans to resolve them: Nothing to report.
Changes that had a significant impact on expenditures: Nothing to report.
Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to report.

Products

Journal publications:

Bharat A, **Bhorade SM**, Morales-Nebreda L, McQuattie-Pimentel AC, **Soberanes S**, Ridge K, DeCamp MM, Mestan KK, **Perlman H**, **Budinger GR**, **Misharin AV**. Flow Cytometry Reveals Similarities Between Lung Macrophages in Humans and Mice. Am J Respir Cell Mol Biol. 2016 Jan;54(1):147-9. doi: 10.1165/rcmb.2015-0147LE. Published. Federal support acknowledged.

Presentations:

1. Keystone Symposia meeting on Epigenetic and Metabolic Regulation of Aging and Aging-Related Diseases. May 1–5, 2016, Santa Fe, USA
Poster: **Transcriptional Profiling Reveals an Aging Signature in the Mouse Lung that Persists During Influenza Infection**
James M Walter, Alexandra Mc Quattie-Pimentel, Paul Reyfman, Francisco Gonzalez, Harris Perlman, GR Scott Budinger, Alexander V Misharin
2. American Thoracic Society Meeting, May 13–18, 2016, San Francisco, USA.
Talk: **Macrophage Heterogeneity in the Aging Lung**
GR Scott Budinger.
3. American Thoracic Society Meeting, May 13–18, 2016, San Francisco, USA.
Talk: A2626 **Transcriptional Profiling Reveals an Aging Signature that Persists During Influenza Infection**
PA Reyfman, S Berdnikovs, AC McQuattie-Pimentel, FJ Gonzalez-Gonzalez, M Chi, NS Chandel, H Perlman, KM Ridge, WE Balch, RI Morimoto, JI Sznajder, GR Scott Budinger, AV Misharin.
4. American Thoracic Society Meeting, May 13–18, 2016, San Francisco, USA.
Talk: **Monocyte-Derived Alveolar Macrophages Contribute to the Development of Lung Fibrosis and Persist in the Lung Over the Lifespan**
AV Misharin.

Participants and other collaborating organizations

Name: **GR Scott Budinger**
Project Role: Principal Investigator
Researcher Identifier: NA
Nearest person month worked: 1.2 CM
Contribution to Project: Dr. Budinger has prepared IRB protocol and worked on its revisions. He supervised generation of the shielded bone marrow chimeras and development of the new fate-mapping mouse models. He performed acquisition of the human lung samples for FACSorting, interpretation of RNA-seq results and presented data at the conferences.

Name: **Alexander Misharin**
Project Role: Partnering Principal Investigator
Researcher Identifier: orcid.org/0000-0003-2879-3789
Nearest person month worked: 3.6 CM
Contribution to Project: Dr. Misharin has prepared animal protocol and worked on its revisions. He generated shielded bone marrow chimeras and worked on generating new fate-mapping mouse models. He performed processing and FACSorting of the human lung specimens for the population and single cell RNA-seq and library construction for RNA-seq, RNA-seq, data processing and interpretation. He authored manuscript and presented data at the conferenses.

Name: **Ching-I Chen**
Project Role: Postdoctoral Researches
Researcher Identifier: NA
Nearest person month worked: 12 CM
Contribution to Project: Dr. Chen monitored shielded bone marrow chimeras, administered influenza virus and bleomycin, performed cell sorting, managed mouse colony and worked on generating new fate-mapping mouse models.

Name: **Saul Soberanes**
Project Role: Postdoctoral Researches
Researcher Identifier: NA
Nearest person month worked: 3 CM
Contribution to Project: Dr. Soberanes monitored shielded bone marrow chimeras, managed mouse colony and worked on generating new fate-mapping mouse models.

Name: **Raul Piseaux**
Project Role: Postdoctoral Researches
Researcher Identifier: NA
Nearest person month worked: 12 CM
Contribution to Project: Dr. Piseaux monitored shielded bone marrow chimeras, managed mouse colony and worked on generating new fate-mapping mouse models.

Name: **Hiroaki Matsuda**
Project Role: Postdoctoral Researches
Researcher Identifier: NA

Nearest person month worked: 3 CM
Contribution to Project: Dr. Matsuda has been working on developing bioinformatics pipelines for analysis of the population-based and single cell RNA-seq data. Dr. Matsuda coordinates monthly bioinformatics meetings for our group.

Name: **Sergejs Berdnikovs**
Project Role: Co-Investigator
Researcher Identifier: NA
Nearest person month worked: 0.6 CM
Contribution to Project: Dr. Berdnikovs provided his expertise in transcriptomic analysis of the population-based (mouse and human) and single cell RNA-seq (human) data.

Name: **Elizabeth Bartom**
Project Role: Co-Investigator
Researcher Identifier: NA
Nearest person month worked: 1.2 CM
Contribution to Project: Dr. Bartom developed and continuing to provide support to bioinformatics pipelines for analysis of the population-based and single cell RNA-seq data. She plays crucial role in integrating our sequencing pipeline into current computational infrastructure at Northwestern (Hundred Genomic Nodes/QUEST project).

Name: **Sangeeta Bhorade**
Project Role: Co-Investigator
Researcher Identifier: NA
Nearest person month worked: 0.6 CM
Contribution to Project: Dr. Bhorade coordinate recruitment of the human subjects into lung transplant program and provided us with invaluable human samples. She is one of the first co-authors our manuscript on flow cytometric analysis of macrophages in the human lung.

Name: **Gokhan Mutlu**
Project Role: Co-Investigator
Researcher Identifier: NA
Nearest person month worked: 1.2 CM
Contribution to Project: Dr. Mutlu coordinated recruitment of the human subjects into lung transplant program (University of Chicago site) and provided us with invaluable human samples.

Name: **Harris Perlman**
Project Role: Co-Investigator
Researcher Identifier: NA
Nearest person month worked: 0.12 CM
Contribution to Project: Dr. Perlman provided his expertise in macrophage biology and helped us to design and set up animal experiments and to manage our conditional knockout strains. He co-authored on our manuscript on flow cytometric analysis of macrophages in the human lung.

Special reporting requirements

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

There are following updates for the other support:

Dr. Budinger:

- The project "Signaling in the lung induced by particulate matter air pollution (R01ES013995)" ended on 4/30/2016
- The project "Pathophysiology of acute lung injury-Core C: Murine Genetics and Phenotyping Core (P01HL071643)" was extended to 6/30/2020
- The project "Disordered Proteostasis as a Driver of Disease in Aging Lung (P01AG049665)" was funded to support 4.2 CM effort.

Dr. Misharin:

- The project "Therapeutic Role for P21 in Suppressing IL-1 Beta Mediated Pathologies" (R01AR050250) ended on 4/30/15.
- The project "Disordered Proteostasis as a Driver of Disease in Aging Lung (P01AG049665)" was funded to support 4.8 CM effort.

Those updates have no impact on the efforts of this project

Other organizations involved as partners:
Organization Name: University of Chicago, Section of Pulmonary and Critical Care Medicine, Dr. Gokhan Mutlu
Location of Organization: Chicago, IL
Partner's contribution to the project: Dr. Mutlu provided us with human lung specimens for to fulfill the research as described in Aim 3.
Financial support: not applicable

Organization Name: Weizmann Institute of Science, Dr. Ido Amit
Location of Organization: Rehovot, Israel
Partner's contribution to the project: Dr. Amit shared with us his expertise in single cell RNA-seq (MARS-seq protocol), provided us detailed protocols and initial set of reagents (capture plates) for single cell RNA-seq and assisted us with preliminary data analysis.
Financial support: not applicable

Appendices

Nothing to report.