AWARD NUMBER: W81XWH-17-1-0503

TITLE: Constrictive Bronchiolitis in Previously Deployed Soldiers

PRINCIPAL INVESTIGATOR: Dr. Vasiliy Polosukhin, MD, PhD

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Nashville, TN 37232-0011

REPORT DATE: October 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

34102

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE: Oct 2019	**2. REPORT TYPE:** Annual	**3. DATES COVERED** 09/15/2018 – 09/14/2019

4. TITLE AND SUBTITLE: Constrictive Bronchiolitis in Previously Deployed Soldiers

5a. CONTRACT NUMBER

5b. GRANT NUMBER W81XWH-17-1-0503

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT We performed histopathological analysis of small airways (bronchioles) from 27 soldiers with constrictive bronchiolitis (CB), 55 patients with chronic obstructive pulmonary disease (COPD) and 18 non-diseased non-smoking (NS) controls. We found widespread pathological changes in COPD airways (loss of alveolar attachments, wall thickening and remodeling, decreased collagen density, and luminal occlusion by mucus plugs. Small airways of soldiers with CB were characterized by concentric fibrosis with increased collagen density and reduced elastin density without increase of wall thickness. These data provide reasonable explanation why soldiers with CB do not develop airway obstruction in spite of advanced fibrous remodeling in their small airways. Soldiers with CB also showed increased wall-to-lumen diameter ratio and small muscle hypertrophy in distal arterioles, collagen and elastin deposition and reduced density of blood capillaries in interalveolar septa, and thickening and fibrosis of pleura suggesting that pathological changes develop in all lung tissue compartments. We performed additional experiments in which we increased sulfur dioxide dose and found out that inhalation exposure (125 ppm) for 4 hours every day for 2 weeks is optimal. Based on similarity of pathological changes in exposed mice with those in soldiers with CB, we selected this model for future work.

15. SUBJECT TERMS Soldiers, constrictive bronchiolitis, small airways, airway inflammation, airway remodeling, airway epithelium, p73, polymeric immunoglobulin receptor, secretory IgA, mice, sulfur dioxide.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	19	

Table of Contents

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Constrictive bronchiolitis (CB) is a rare lung disease characterized by fibrotic remodeling of small airways. In 2011, King and colleagues reported that pathologic findings consistent with constrictive bronchiolitis were present in 38 active-duty military personnel presenting for evaluation for exertional dyspnea. All soldiers had previously been deployed to Afghanistan and/or Iraq as part of Operation Enduring Freedom or Operation Iraqi Freedom (OEF/OIF), and many of them reported exposure to the sulfur fire at the Mishraq State sulfur mine in June 2003. While certain inhalational exposures have previously been associated with constrictive bronchiolitis, the mechanisms responsible for initiation and progression of the disease after inhalational injury are unknown. We hypothesize that exposure to airborne biohazards/toxins causes direct injury to the bronchiolar epithelium with loss of p73 expression, which is required for maintenance of the multi-ciliated cell (MCC) phenotype. Loss of MCCs leads to a reduction in pIgR, resulting in surface secretory IgA deficiency and a defective epithelial immune barrier that allows bacteria and inhaled antigens to drive persistent inflammation and fibrous remodeling of these small airways. To test our hypothesis, we propose the following specific aims: 1) to analyze the relationship between aberrant epithelial differentiation, loss of epithelial immune barrier function, and mural inflammation/remodeling in constrictive bronchiolitis; and 2) to investigate sulfur dioxide inhalation as a model of constrictive bronchiolitis in mice and test potential interventions. Our studies will investigate the novel concept that abnormal epithelial differentiation and impaired mucosal immunity underlie the pathogenesis of this disease, and will be among the first studies to investigate the role of p73 transcription factor in airway disease. In addition, our studies will characterize a new murine model of constrictive bronchiolitis that can be used to investigate potential therapeutics for this important disease.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

soldiers, constrictive bronchiolitis, small airways, airway inflammation, airway remodeling, airway epithelium, p73, polymeric immunoglobulin receptor, secretory IgA, mice, sulfur dioxide.

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

	Timeline (months)	**Percentage of completion**
Specific Aim 1: To analyze the relationship between aberrant epithelial differentiation, loss of epithelial immune barrier function and mural inflammation/remodeling in constrictive bronchiolitis.		
Local IRB approval: IRB approval is anticipated prior to or shortly after the beginning of the study period.	1-6	Approval received
Milestone(s) Achieved: USAMRMC Human Research Protection Office approval.	6	Approval received
Task 1: Evaluate lung samples from patients with constrictive bronchiolitis for epithelial structural changes, inflammation, and airway wall remodeling	1-18	
Subtask 1.1: Histological analysis of bronchiolar epithelial structural changes in constrictive bronchiolitis (e.g. immunohistochemical detection and count of ciliated cells, club cells and undifferentiated cells).		Complete
Subtask 1.2: Histological analysis of bronchiolar fibrous remodeling in constrictive bronchiolitis (e.g. quantitative measurements of wall thickening using histomorphometry technique).		Complete
Subtask 1.3: Histological analysis of bronchiolar inflammation in constrictive bronchiolitis (e.g. immunohistochemical detection and quantification of neutrophils, CD4, CD8 and CD19 lymphocytes).		Complete
Subtask 1.4: Protein isolation from frozen lung tissue samples and measurements of pro-inflammatory cytokines.		20%
Subtask 1.5: RNA isolation from frozen lung tissue samples and analysis of pro-inflammatory cytokine gene expression.		Complete
Milestone(s) Achieved: Evaluation of histopathological changes in individual bronchioles of soldiers with constrictive bronchiolitis, comparison analysis among study groups and data documentation.	18	90%

Task 2: Define epithelial immune barrier dysfunction in respiratory bronchioles in constrictive bronchiolitis	12-24	
Subtask 2.1: Detection and quantification of SIgA on surface of individual bronchioles in constrictive bronchiolitis (e.g. immunohistochemical detection and quantification of pIgR-positive ciliated cells in epithelial lining, direct measurement of IgA-specific immunofluorescence signal on mucosal surface of individual bronchioles).		Complete
Subtask 2.2: Detection and quantification of NF-κB signaling in bronchiolar epithelial cells (e.g. immunohistochemical detection and count of phospho-p65-positive cells).		50%
Milestone(s) Achieved: Histological evaluation of mucosal immune disorders in individual bronchioles in constrictive bronchiolitis.	24	75%
Task 3: Investigate bacterial invasion of the epithelial barrier in constrictive bronchiolitis.	18-36	
Subtask 3.1: Detection of bacterial species in individual bronchioles using *in situ* hybridization technique.		50%
Subtask 3.2: DNA isolation from frozen lung samples and detailed analysis of lung microbiome using Respiratory Infections Microbial DNA qPCR Array.		20%
Milestone(s) Achieved: Analysis of microbiome alterations in the lungs of soldiers with constrictive bronchiolitis (demonstration whether a more invasive or pro-inflammatory microbiome can be detected in the lungs of soldiers with constrictive bronchiolitis).	36	30%
Specific Aim 2: To investigate sulfur dioxide inhalation as a model of constrictive bronchiolitis in mice and test potential interventions.		
Local IACUC approval: IACUC approval is anticipated prior to or shortly after the beginning of the study period.	1-3	Approval received
Milestone(s) Achieved: USAMRMC Animal Care and Use Review Office approval.	3	Approval received
Task 1: Optimizing our model of sulfur dioxide exposure (SO_2) to generate constrictive bronchiolitis. Total 96 mice will be used.	1-12	

Subtask 1.1: Mouse ordering and colony breeding.		Complete
Subtask 1.2: Inhalation exposure of mice to SO_2 using two different scheme: 1) single exposure to 125 ppm SO_2 for 4 hours and 2) repetitive exposures to 125 ppm SO_2 for 2 hours every other day for 1 week or 2 weeks.		Complete
Subtask 1.3: Analysis and quantification of fibrous remodeling in distal airways in mice after single or repetitive SO_2 inhalation exposure.		Complete
Subtask 1.4: Analysis of airway epithelial cell injury and regeneration/differentiation in mice after single or repetitive SO_2 inhalation exposure.		50%
Subtask 1.5: Analysis of airway epithelial immune barrier dysfunction in mice after single or repetitive SO_2 inhalation exposure.		50%
Subtask 1.6: Analysis of lung inflammation in mice after single or repetitive SO_2 inhalation exposure.		50%
Subtask 1.7: Analysis of differences in lung microbiome in mice after single or repetitive SO_2 inhalation exposure.		0%
Milestone(s) Achieved: Evaluation of pathological changes in distal airways in mice after inhalation exposure to SO_2. Optimization of mouse model of constrictive bronchiolitis for future studies.	12	80%
Task 2: Time course study of airway pathology in mice after inhalation exposure to SO_2. Total 88 mice will be used.	12-24	
Subtask 2.1: Time course study (2, 4 or 6 months) for dynamic assessment of lung inflammation and airway fibrous remodeling in mice after inhalation exposure to SO_2. Total 48 mice will be used.		0%
Subtask 2.2: Time course study (48 hours, 1 week, 1 or 2 months) for analysis of ultrastructural manifestations of lung cell/tissue injury and airway fibrous remodeling developed in response to inhalation exposure to SO_2. Total 40 mice will be used.		0%

Milestone(s) Achieved: Dynamic analysis of histological and ultrastructural manifestations of pathological changes in distal airways in mice after inhalation exposure to SO_2.	24	0%
Task 3: Determining whether SO_2 exposure affects differentiation of ciliated cells *ex vivo*. Total 240 mice will be used.	6-24	
Subtask 3.1: Analysis of ability of tracheal epithelial cells to restore differentiation and structural specificity *ex vivo* after SO2 exposure.		0%
Subtask 3.2: Determining whether overexpression of p73 or FoxJ1 transcription factors can improve tracheal epithelial cell differentiation *ex vivo* after SO2 exposure.		0%
Milestone(s) Achieved: The role of transcription factors p73 and FoxJ1 in post-injurious regeneration and differentiation of airway epithelial cell will be investigated.	24	0%
Task 4: Interventions to prevent airway fibrosis in mice. Total 160 mice will be used.	25-36	
Subtask 4.1: Determining whether roflumilast intervention can prevent airway fibrosis in mice after SO2 inhalation exposure.		0%
Subtask 4.2: Determining whether antibiotics intervention can prevent airway fibrosis in mice after SO2 inhalation exposure.		0%
Milestone(s) Achieved: The test of hypothesis that anti-inflammatory treatments would be beneficial for lung inflammation and airway fibrosis.	36	0%

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

Specific Aim 1: To analyze the relationship between aberrant epithelial differentiation, loss of epithelial immune barrier function and mural inflammation/remodeling in constrictive bronchiolitis.

At the second year of work, we:

1) evaluated histopathological changes in the lungs of soldiers with constrictive bronchiolitis (CB) in comparison with patients with chronic obstructive pulmonary disease (COPD) in comparison to non-diseased non-smoking (NS) controls;
2) performed analysis of pro-inflammatory gene expression in the lungs of soldiers with CB and non-diseased NS controls.

Task 1: Evaluate lung samples from patients with constrictive bronchiolitis for epithelial structural changes, inflammation, and airway wall remodeling

Subtask 1.1: Histological analysis of bronchiolar epithelial structural changes in constrictive bronchiolitis (e.g. immunohistochemical detection and count of ciliated cells, club cells and undifferentiated cells).

Completed in the first year.

Subtask 1.2: Histological analysis of bronchiolar fibrous remodeling in constrictive bronchiolitis (e.g. quantitative measurements of wall thickening using histomorphometry technique).

In the first and the second years, we performed histological and morphometrical analyses of small airways (bronchioles) from 27 soldiers with CB, 55 patients with COPD and 18 non-diseased NS controls in order to determine the factor(s) that drive airflow limitation. We assessed small airways for alveolar attachments, wall remodeling/thickening, collagen/elastin density and luminal mucus plugging. Small airways from COPD patients showed complex pathological changes including loss of alveolar attachments, wall thickening and remodeling, decreased collagen density, and frequent luminal occlusion by mucus plugs. Small airways of soldiers with CB were characterized by concentric fibrosis with increased collagen density and reduced elastin density without increase of wall thickness. Alveolar attachments and luminal mucus were similar between CB and NS airways. Since soldiers with CB do not develop airway obstruction, we suggest that isolated small airway pathology (fibrous remodeling) detected in their small airways is insufficient to cause significant airflow limitation; whereas, combined pathology (wall thickening, collagen scaffold degradation, loss of alveolar attachments and luminal mucus plugging) affects air flow through small airways in patients with COPD. Multivariable linear regression analysis revealed that loss of alveolar attachments is the major determinant of airflow limitation in COPD. Together, our data provide reasonable explanation why soldiers with CB do not develop airway obstruction in spite of advanced fibrous remodeling in their small airways. Results of this work have been submitted for publication in American Journal of Respiratory and Critical Care Medicine.

When we examined tissue samples, we have found that soldiers with CB develop pathological changes in other lung compartments including distal arterioles, alveolar tissue and pleura. While these works were not proposed in approved SOW, we decided to increase soldier's group up to 40 and performed comprehensive histomorphometry for small airways, distal arterioles, and alveolar tissue. In comparison to NS controls, soldiers with CB showed significant collagen deposition, reduced elastin content and smooth muscle hypertrophy in bronchiolar walls (Figure 1), increased wall-to-lumen diameter ratio and small muscle

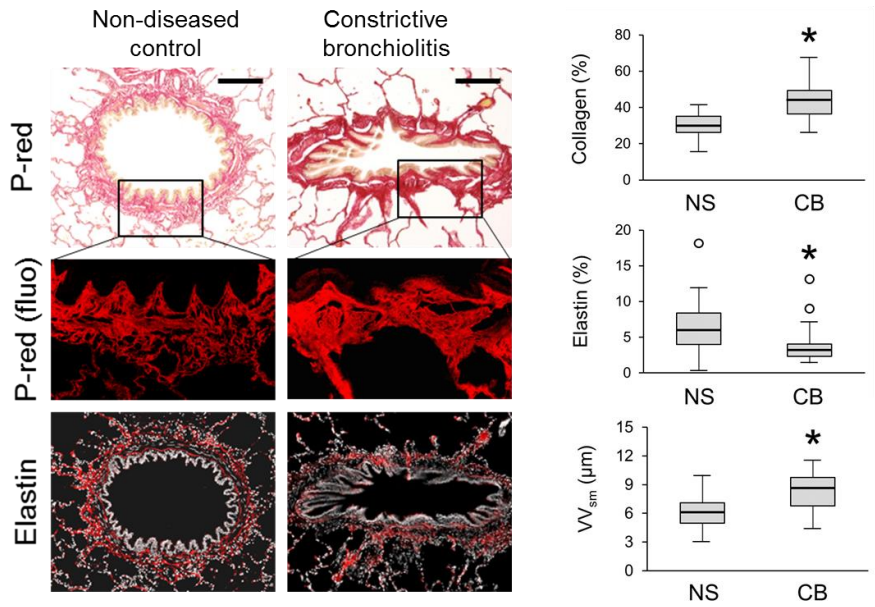

Figure 1. Increased collagen content, decreased elastin content and smooth muscle hypertrophy in small airway walls from soldiers with CB. VV_{sm} – smooth muscle volume density, a parameter showing smooth muscle hypertrophy.

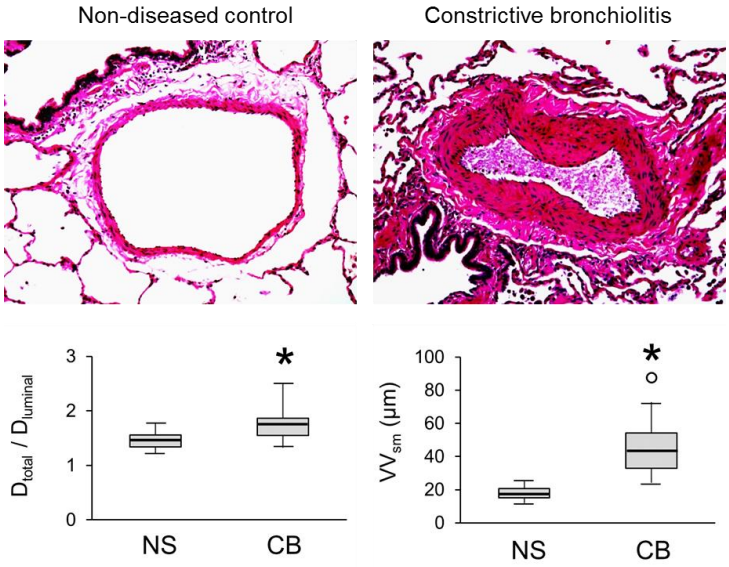

Figure 2. Vascular pathology in pulmonary arterioles from CB patients. D_{total} / $D_{luminal}$ – wall-to-lumen diameter ratio; VV_{sm} – smooth muscle volume density, a parameter showing smooth muscle hypertrophy.

hypertrophy in distal arterioles (Figure 2), collagen and elastin deposition and reduced density of blood capillaries in interalveolar septa (Figures 3 and 4), thickening and fibrosis of pleura. Together, these data suggest that pathological changes develop in all lung tissue compartments.

To complete this work, we collected tissue samples from 6 civilians with post-transplant CB (hematopoietic stem cell transplantation) and 15 patients with pulmonary arterial hypertension. We believe that this extensive study will be helpful to understand the nature of exertional dyspnea in soldiers with deployment-related CB.

Subtask 1.3: Histological analysis of bronchiolar inflammation in constrictive bronchiolitis (e.g. immunohistochemical detection and quantification of neutrophils, CD4, CD8 and CD19 lymphocytes).

We quantified immune inflammatory cells in bronchiolar walls and detected significant accumulation of CD4 and CD8 T lymphocytes and CD19 B lymphocytes in soldiers with CB compared to non-diseased NS controls. The number of neutrophils was low and indistinguishable from airways of control subjects. In contrast, we showed similar levels of

lymphocyte (CD4, CD8 and CD19-positive lymphoid follicles) accumulation in small airways in soldiers with CB and patients with COPD.

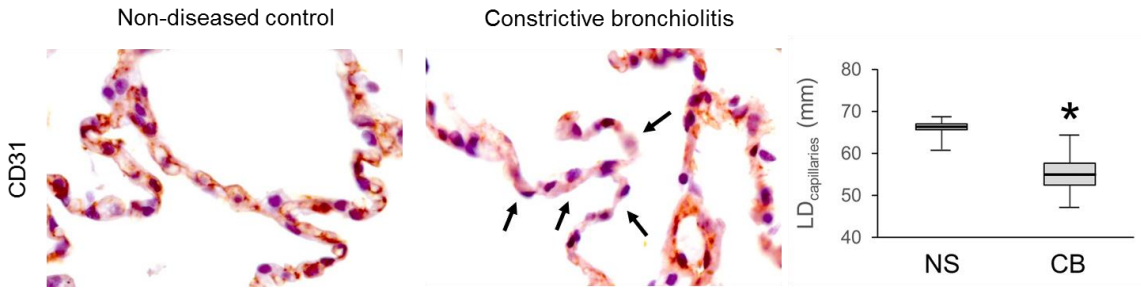

Figure 3. Capillary loss in interalveolar septa in soldiers with CB (arrows). $LD_{capillaries}$ – linear density of blood capillaries in interalveolar septa (number of blood capillaries per 1 mm of interalveolar septal length.

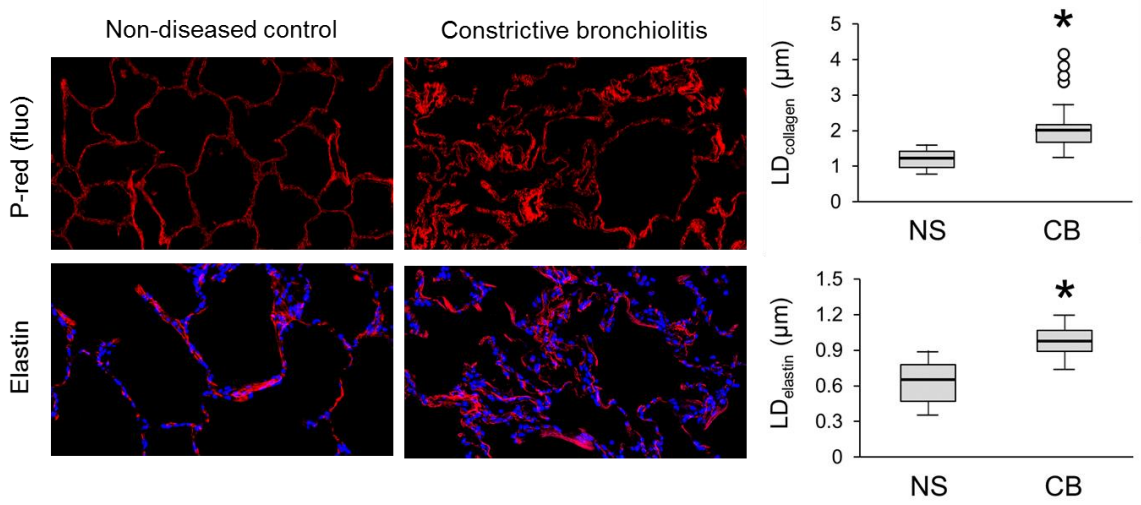

Figure 4. Fibrous remodeling of interalveolar septa in soldiers with CB. LD – linear density of collagen or elastin, a parameter showing collagen/elastin content (collagen/elastin fractional area in alveolar tissue normalized to 1 mm of interalveolar septal length).

In COPD patients, we found a close correlation between neutrophil invasion into the airway wall and alveolar attachment loss, implicating these cells as the likely mediators of attachment destruction. These findings are further supported by our findings in non-obstructed CB patients, where neutrophil influx was not observed and alveolar attachments were intact. Since increased numbers of T cells and lymphoid aggregates are present in and around diseased airways in soldiers with CB, it is tempting to speculate that these immune cells contribute to the fibrotic remodeling present in these airways.

Subtask 1.4: Protein isolation from frozen lung tissue samples and measurements of pro-inflammatory cytokines.

We isolated proteins from available lung tissue samples from patients with COPD and non-diseased NS controls. This preliminary work is necessary to establish protocol for future studies.

Subtask 1.5: RNA isolation from frozen lung tissue samples and analysis of pro-inflammatory cytokine gene expression.

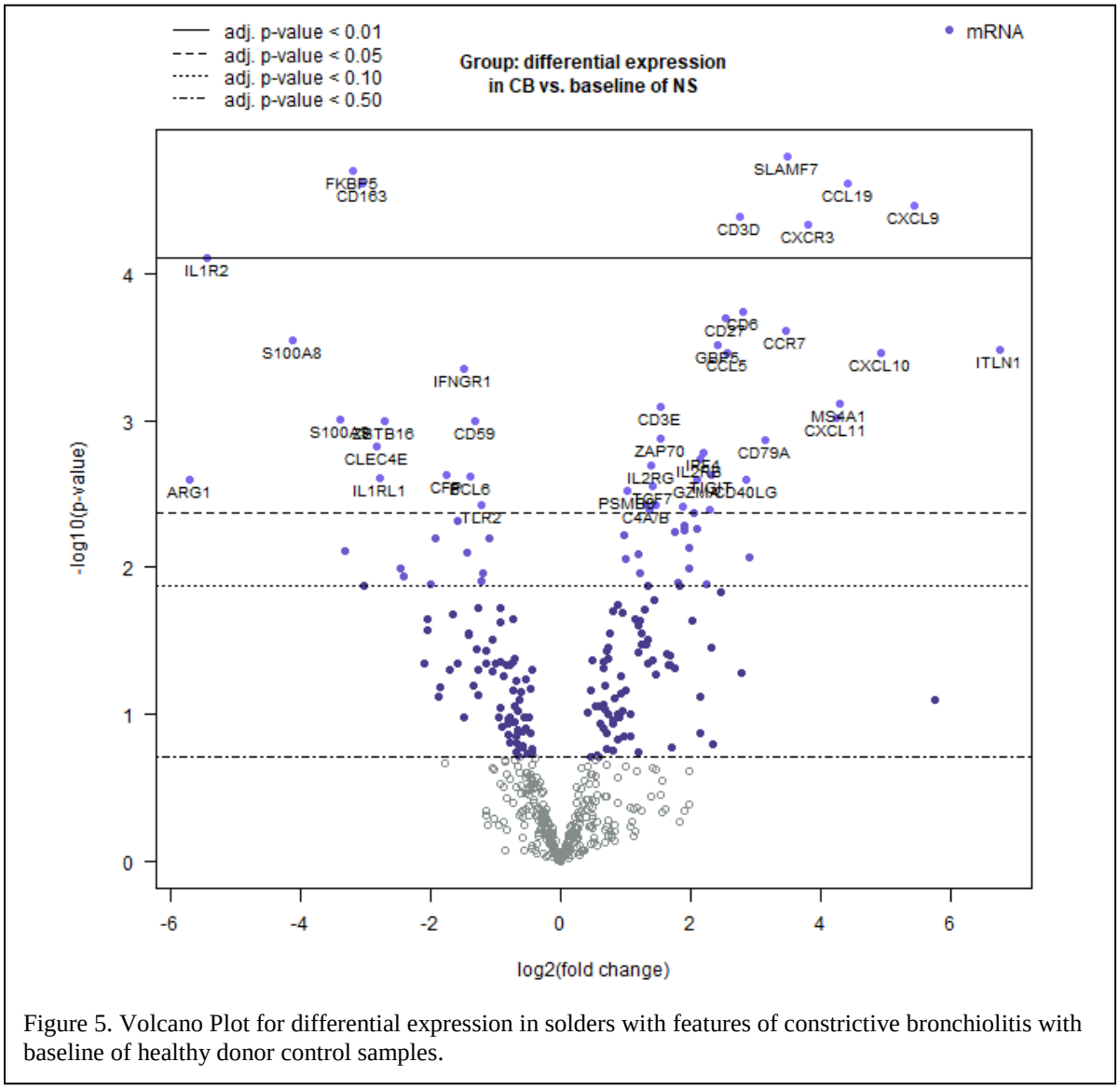

Figure 5. Volcano Plot for differential expression in solders with features of constrictive bronchiolitis with baseline of healthy donor control samples.

We isolated RNA from 8 patients with CB and 4 non-diseased NS controls. For RNA expression analysis, we used nCounter® Immunology Panel (NanoString Technologies, WA). The panel is based on a novel digital molecular barcoding technology (PlexSet Technology) that allows to count directly the RNA molecules. With this panel, we performed multiplex gene expression analysis for 579 immune inflammatory genes and 15 internal reference genes. This panel includes major classes of cytokines and their receptors, enzymes with specific gene families such as the major chemokine ligands and receptors, interferons

and their receptors, the TNF-receptor superfamily, and the KIR family genes. 84 genes involved with the anti-fungal immune response are also included.

The differential expression analysis in soldier's samples with baseline from NS controls with Benjamini-Hochberg (BH) p-adjustment revealed 45 genes with at least 2-fold change in expression (Figure 5).

The Reactome Project analysis revealed up-regulation of genes responsible for:

1. adaptive immune pathways including B Cell Receptor (BCR)-signaling, T Cell Receptor (TCR)-signaling, immunoregulatory interactions between Lymphoid and non-Lymphoid cells, co-stimulation by the CD28 family;
2. cytokine signaling including TNFR2 non-canonical NF-kB pathway, Interleukin-2 family signaling and Interleukin-3, Interleukin-5 and GM-CSF signaling, interferon gamma signaling;
3. RAF/MAP kinase cascade;
4. signal transduction including signaling by GPCR, signaling by WNT, regulation of KIT signaling.

In contrast, genes participating in innate immune response associated pathways such as Toll-like Receptor cascades, neutrophil degranulation, TRAF6 mediated NF-kB activation, complement cascade, antimicrobial peptides, advanced glycosylation end product receptor signaling are down-regulated.

In addition, genes participated in following pathways are down-regulated:

1. Interleukin-1 family signaling;
2. signal transduction including signaling by Rho GTPases;
3. gene expression (transcription) including TP53 Regulates Transcription of Cell Death Genes, FOXO-mediated transcription of cell death genes, MECP2 regulates neuronal receptors and channels from RNA Polymerase II Transcription.

Interestingly, Interleukin-4 and Interleukin-13 signaling and Interleukin-10 signaling pathways contained both up-regulated and down-regulated genes.

Together, our findings related to **Task 1** show pathological changes in all analyzed lung tissue compartments. Persistent accumulation of lymphocytes suggests an ongoing local activation of adaptive immunity. It is important to note that observed selective up-regulation of genes responsible for adaptive immune response has close relationship to histopathological changes.

Task 2 and 3:

In the first year, we have measured IgA-specific immunofluorescence on the epithelial surface of bronchioles from 14 soldiers with constrictive bronchiolitis and 8 non-diseased lifelong non-smokers and 10 former smokers without small airway disease. Significant reduction of IgA immunofluorescence was detected on mucosal surface of bronchioles of soldiers suggesting mucosal SIgA deficiency.

Our work in the second year showed that SIgA deficiency in soldiers with CB is associated with incomplete ciliated cell differentiation in bronchiolar epithelium; whereas, SIgA deficiency in patients with COPD is associated with altered epithelial cell differentiation towards goblet cell metaplasia or stratification. These results further support our hypothesis that epithelial alterations and immune barrier distortions play a role in the pathogenesis of small airways diseases such as CB and COPD.

Specific Aim 2: To investigate sulfur dioxide inhalation as a model of constrictive bronchiolitis in mice and test potential interventions.

At the second year of work, we:
1) established mouse model of CB.
2) Performed initial histological and morphometrical analysis of mouse lung specimens.

Task 1: Optimizing our model of sulfur dioxide exposure (SO_2) to generate constrictive bronchiolitis.

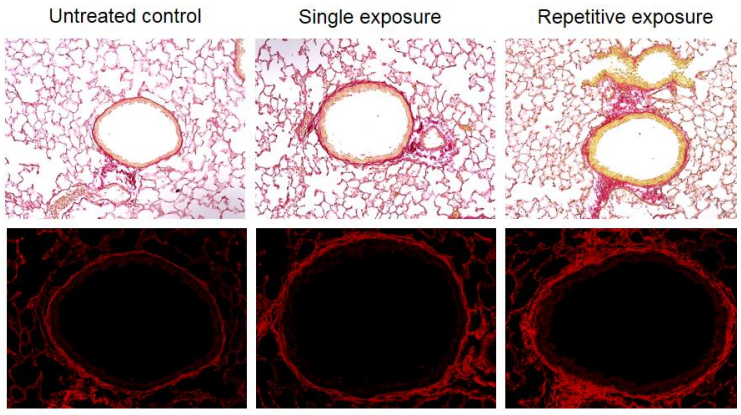

Figure 6. *Sulfur dioxide inhalation causes persistent fibrotic remodeling of small airway walls.* An untreated control mouse shows normal structure of airway wall and fine structural organization of collagen fibers in subepithelium. Mice, harvested at 2 months after a single 4-hour exposure to SO_2 at 125 ppm or repetitive 2-hour exposure to SO_2 at 125 ppm every other day for 2 weeks, show dose dependent airway wall fibrous remodeling. No inflammation or fibrosis is observed in lung parenchyma after SO_2 exposure. Top row – staining Picrosirius red; bottom row – red fluorescence of collagen fibers.

Subtask 1.2: Inhalation exposure of mice to SO_2 using two different schemes: 1) single exposure to 125 ppm SO_2 for 4 hours and 2) repetitive exposures to 125 ppm SO_2 for 2 hours every other day for 1 week or 2 weeks.

In the first year, we found out that repetitive inhalation exposure of mice to SO_2 showed more prominent epithelial changes (loss of ciliated cells) and fibrosis of distal airways than after single treatment (Figure 6). Although repetitive SO_2 exposure appears to cause more substantial fibrotic airway wall remodeling than single exposure, both models cause only mild remodeling of small airways.

During the second year, we performed additional experiments in which we increased SO_2 dose. We found out that inhalation exposure (125 ppm) for 4 hours every day for 2 weeks is optimal. The pathological changes detected in exposed mice include airway fibrous remodeling, smooth muscle hypertrophy in distal arterioles and collagen accumulation within interalveolar septa. Based on similarity of pathological changes in exposed mice with those in soldiers with CB, we selected this model for future work.

Subtasks 1.3-1.5 and 2.1-2.2:

We analyzed and quantified fibrotic manifestations of distal airways in mice after single or repetitive SO_2 inhalation exposure. We performed immunostainings for alpha-tubulin for ciliated cell quantification; for neutrophil elastase and CD3 for neutrophil and lymphocyte quantification; for IgA and polymeric immunoglobulin receptor for SIgA quantification on airway mucosal surface.

- **What opportunities for training and professional development has the project provided?**

  Nothing to report.

- **How were the results disseminated to communities of interest?**

  Nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

  We are close to completion of dataset with histological and morphometrical assessment of small airways, arterioles, alveolar tissue and pleura from US soldiers in comparison with patients with post-transplant CB, patients with PAH and non-diseased NS subjects. We expect to submit these results for publication in early 2020.

  We found that SO_2 inhalation results in lung pathology in mice that recapitulates general manifestations detected in soldiers with CB (small airway fibrosis and remodeling, smooth muscle hypertrophy in distal arterioles and collagen deposition within interalveolar septa). Further studies will be performed as described in the SOW.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

  Constrictive bronchiolitis is an emerging health problem in US soldiers, and is an important cause of disability in this population. Our studies investigate the novel concept that abnormal epithelial differentiation and impaired mucosal immunity underlie the pathogenesis of this disease. In addition, our studies characterize a new murine model of constrictive bronchiolitis that can be used to investigate potential therapeutics for this important disease.

- **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:**

Since we have detected more prominent pathological changes in the lungs of soldiers with CB than initially assessed, we decided to perform additional histological and morphometrical analyses. We also decided to include additional study groups (civilians with post-transplant CB and patients with PAH) to make our further study more detailed and objective. While this additional works will slow down approved SOW, we believe this would be beneficial for the whole project and potentially lead to better understanding of pathogenesis of post-deployment CB.

- **Changes in approach and reasons for change**

See above.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

See above.

- **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**
- **Significant changes in use or care of human subjects**
- **Significant changes in use or care of vertebrate animals.**

We have approved an amendment to our current IACUC protocol for the additional experiments proposed with increased dose and/or duration of SO_2 exposure.

- **Significant changes in use of biohazards and/or select agents**

Nothing to report.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**

Results were presented:

1. American Thoracic Society 2019 International Conference, May 17-22, 2019, Dallas, Texas. Title of presentation: "The Histopathological Basis of Airflow Limitation in Small Airways Disease".

2. International Congress of Mucosal Immunology, July 16-20, Brisbane, Australia. Title of presentation: "Mucosal Immune Disorders in Small Airways Disease".

- **Journal publications.**

  In progress.

- **Books or other non-periodical, one-time publications.**

  1. Gutor S, Du R-H, Miller RF, Richmond BW, Massion PP, Blackwell TS, Polosukhin VV. The Histopathological Basis of Airflow Limitation in Small Airways Disease. *Am J Respir Crit Care Med* 2019;199:A5784 (abstract)**.**

- **Other publications, conference papers, and presentations.**

  Nothing to report

- **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:	Vasiliy V. Polosukhin, MD, PhD
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	8.4
Contribution to Project:	Dr. Polosukhin has performed completion of the work proposed, experiment planning, interpreting and trouble-shooting, and facilitated interactions among investigators.
Funding Support:	No change.

Name:	Sergey S. Gutor
Project Role:	Postdoctoral Research Fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	12
Contribution to Project:	Dr. Gutor preforms histopathological and morphometrical examination of human and mouse lung tissue samples with the oversight of Dr. Polosukhin.
Funding Support:	No change.

Name:	Pingsheng Wu
Project Role:	Research Assistant Professor
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Wu performed statistical work.
Funding Support:	No change.

Name:	Rui-Hong Du
Project Role:	Research Assistant III
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	9.55
Contribution to Project:	Ms. Du manages the animal colony with the oversight of Dr. Polosukhin. She will also perform immunohistochemistry, in situ hybridization and variety of cell and molecular biology techniques, cell culturing and ELISAs.
Funding Support:	No change.

Name:	Raphael Hunt
Project Role:	Research Assistant III
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3
Contribution to Project:	N/A
Funding Support:	No change.

- **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

  Polosukhin, Vasiliy M.D.
  Ended: None
  New: 1 U01 HL145561-01 (Shaykiev-Weill Cornell)

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

  Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS**
- **COLLABORATIVE AWARDS:**
- **QUAD CHARTS:**

  Nothing to report.

9. **APPENDICES:**

Nothing to report.