AWARD NUMBER: W81XH-21-1-0666

TITLE: Innate Immunity and Deployment-Related Lung Disease

PRINCIPAL INVESTIGATOR: Gregory P. Downey, MD

CONTRACTING ORGANIZATION: National Jewish Health, Denver, CO

REPORT DATE: October 2022

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2022	Annual	30Sep2021-29Sep022

4. TITLE AND SUBTITLE

Innate Immunity and Deployment Related Lung Disease

5a. CONTRACT NUMBER
W81XH-21-1-0666

5b. GRANT NUMBER
PR201498

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Downey, Gregory, P., MD
Rose, Cecile, MD
Chu, Hong Wei, MD
Day, Brian, PhD
Seibold, Max, PhD

E-Mail: downeyg@njhealth.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

National Jewish Health
1400 Jackson St
Denver, CO 80206

8. PERFORMING ORGANIZATION REPORT

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 NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT Since 2001, over 3 million United States military personnel and contractors have been deployed to Southwest Asia and Afghanistan where they have been exposed to airborne particulate matter (PM) arising from a combination of sandstorms, explosive blasts and combat-related dust contaminated with toxic pollutants from local industries, burn pit combustion products, and diesel exhaust. An unknown number of military personnel have returned from deployment with persistent respiratory symptoms that limit their ability to meet physical fitness requirements of active duty service and contribute to long term disability. This application will examine the role of immune cells of the respiratory system in causing deployment related lung disease. We will use existing (banked) biological samples obtained from military personnel previously deployed to Southwest Asia who have ongoing respiratory symptoms after deployment. We will analyze the samples using sophisticated molecular (single cell RNA analysis) and protein level (CyTOF mass cytometry) technique to characterize specific populations of immune cells in the lung and how these differ between previously deployed military personnel and healthy subjects. Once we learn the cellular signaling pathways that are linked to these alterations, we will experimentally test their importance in a culture system being thin slices of human lung obtained from organ donor's unused for transplant. In the system, we can test the effects of drugs and also gene therapies to try and prevent the damage to the lung. The ultimate goal of this proposal is to identify novel therapeutic targets in order to diminish injury to the lung and the long-term respiratory consequences of deployment-related lung disease.

15. SUBJECT TERMS
Airborne particulate matter, deployment lung disease, innate immunity, RNAseq, mass cytometry, respiratory epithelium, bronchiolitis, asthma, viral infection

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	20	

TABLE OF CONTENTS

1. **INTRODUCTION:**

Innate immune cells (macrophages, monocytes, innate lymphoid cells, and respiratory epithelial cells) that reside in the upper and lower respiratory system tract are activated ('primed') by exposure to inhaled silicate-containing PM from Southwest Asia contaminated by environmental toxicants; subsequent viral infection amplifies activation of these cells via a pathway involving IL-1β and the NLRP3 inflammasome leading to release of immune and inflammatory mediators resulting increased airways hyperreactivity and, in some cases, irreversible narrowing of the small airways (bronchiolitis). These immune responses underpin the pathogenesis of deployment-related lung disease (DRLD). In this project, we will develop methods to sample innate immune cells in different compartments within the upper and lower respiratory tract including the mucosal surfaces and adjacent tissues of the nose, large and small airways, and alveolar spaces. We will define the types and activation state of respiratory innate immune cells in military personnel with deployment-related respiratory diseases and compare these to healthy controls.

2. **KEYWORDS:**

Airborne particulate matter, deployment lung disease, innate immunity, RNAseq, mass cytometry, respiratory epithelium, bronchiolitis, asthma, viral infection

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

What was accomplished under these goals?

Major Task 1: Determine the cellular and molecular alterations in distal lung innate immune function that occur in DRLD in immune cells within the lower airways and alveoli.

- **Subtask 1.** Local IRB Approval for use of human cells, human precision cut lung slices, and collection of nasal brushings.
    - We received local IRB approval from Biomedical Research Alliance of New York (BRANY) in August 2021 under protocol number HS-3022-528. This included revising a currently existing protocol to allow for the collection of cells via sputum induction among study participants with deployment-related lung disease as well as healthy controls, with oversight/input from our project officers. Screening questions for healthy

controls as well as informed consent documents for study participants with lung disease and controls were approved.
    - We received Continuing Review approval for collection of human induced sputum cells and use of banked epithelial cells from nasal brushings for this study from BRANY in September 2022. All related Continuing Review documents were submitted to HRPO (now the Office of Human Research Oversight, OHRO) in September 2022.
    - We obtained approval from our National Jewish Health/BRANY IRB to use de-identified explanted human donor lungs for experimental use under the category of non-human subjects research. The application to use cadaveric human explanted donor lung tissue has been submitted to OHRO.
- **Milestone Achieved: OHRO submission and approval for the collection of cells via sputum** induction among study participants with deployment-related lung disease as well as healthy controls
    - Submission to OHRO was completed on September 28, 2021, with approval received on November 4, 2021.
    - Continuing Review documents for use of induced sputum samples and banked nasal brushing cells were approved by local IRB and sent for review to OHRO in September 2022.

- **Subtask 2**. Single-Cell RNA-seq analysis of human lung BAL cells
    - We have optimized conditions for the tagging of immune cell samples (including PBMCs) for 10X cell capture, library preparation, and subsequent sequencing. These optimized methods will be used for the BAL samples.
    - As of September 2022 BAL samples from 7 healthy controls have been obtained (from the National Jewish Health institutional Biobank using the Honest Broker system) and cryopreserved for CyTOF, scRNAseq, and bulk expression analyses.

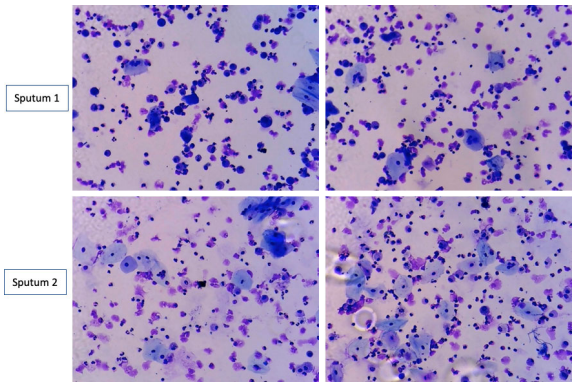
- **Subtask 3.** Mass Cytometry/CyTOF, a single-cell proteomic and immunophenotyping approach.
    - We have collected BAL from research bronchoscopies performed on 37 subjects with deployment-related lung disease. Pilot deployer and control BAL samples were sent to the University of Colorado Anschutz Medical Campus for preliminary flow cytometry (CyTOF) analysis to characterize dominant cell profiles. Additional samples will be sent once additional control samples are collected.
    - **Difficulty encountered:** Collection of healthy control BAL samples was limited during the pandemic.
        - In the past year, we have collected BAL cells from bronchoscopies of healthy control subjects from n=7 subjects for CyTOF analysis.

- **Milestone Achieved**: Single-Cell RNA-seq and mass cytometry efforts are in progress, with proposed goal for additional sample collection and analysis by month 33.

- **Subtask 4.** Prepare and submit manuscripts for publication.
    - Not yet underway (proposed for months 18-36)

Major Task 2: Determine the cellular and molecular level alterations in upper airway innate immune function that occur in DRLD within both the epithelial and upper airway resident immune cell tissue compartments.

- **Subtask 1.** Upper Airway Samples Collected for Analyses
    - We hired and trained a clinical research coordinator to collect high quality induced sputum samples.
    - This clinical research coordinator completed IATA training in specimen handling and shipping as well as CITI training in Biomedical Research, Good Clinical Practice, and Health Information Privacy and Security. Additional training and proficiency was completed on informed consent processes, vital signs, study record-keeping, adverse event reporting, and American Heart Association Basic Life Support.
    - Advanced coordinator training for airway sample collection included formal spirometry instruction and completion of a NIOSH-approved Spirometry and Respiratory Surveillance Training Program as well as sputum induction procedure training requiring administration of nebulized saline and albuterol metered-dose inhalers based on the PrecISE Manual of Operating Procedures (MOP)10 protocol version 1.5 (2/24/2021).
    - We pilot tested sputum cell collection and processing methods to assure specimen quality before beginning study subject recruitment.
        - We piloted sputum sample processing, establishing a system for coordinators to alert lab personnel when specimens have been collected, and then ensure rapid delivery of specimens to the lab. We implemented a collection MOP and processing protocol for these sputum samples. Cytospin cell counts of the two piloted sputum samples revealed a diversity of known airway immune cell types, indicating high quality sample collection and processing.



# of cells scored	Sputum 1	Sputum 2
Squamous (% of view)	15%	10%
Epithelial	1 (0.2%)	2 (0.38%)
Eosinophil	165 (33%)	179 (34.5%)
Monocyte	208 (41.6%)	215 (41.5%)
Lymphocyte	15 (3%)	11 (2.1%)
Neutrophil	112 (22.4%)	111 (21.4%)
TOTAL	**501 (100%)**	**518 (100%)**

Task2.1-Fig. 1: Cell cytospin images generated from samples using our piloted preparation protocol. Cell type differential count results for these samples are shown in the table.

- We created a clinical trial listing (https:/www.nationaljewish.org/clinical-trials/deployment-related-lung-disease-study-healthy-volunteers-needed) to enroll healthy controls for sputum induction sample collection.
- We began healthy control study subject recruitment in September 2022.
- We started recruiting study subjects with deployment-related respiratory diseases, reaching out to military veterans who return for NJH clinical follow-up of their lung diseases.
    - As of September 2022, we have collected sputum from 2 healthy control subjects and 4 deployers.

- Quality control of samples including determination of sample cell type composition was performed.
- Samples were cryopreserved using protocols to allow for later bulk RNAseq and scRNAseq analyses.

- **Subtask 2.** scRNA-seq analysis of airway samples
  - The multiplexing of airway brush cells (nasal and bronchial) has been planned to ensure the best distribution of disease categories across capture sample groups to avoid confounding in our analyses. All required reagents have been ordered and arrived in 9/20222. We recently further refined methods for performing scRNA-seq on frozen nasal and bronchial brushings. All samples are cryopreserved and ready to be processed. Sample capture for scRNAseq will begin in the next quarter.
  - We have optimized methods for scRNA-seq of sputum cell samples using two of the sputum samples collected in the pilot. Cell capture was successful as we generated good quality cDNA, followed by successfully generation of high quality libraries. Sequencing was performed and data is currently being mapped, quantitated, and clustered. Preliminary results indicate we successfully captured 1,619 and 2,024 cells in the dataset. Analyses are currently ongoing.

- **Subtask 3.** Bulk Whole transcriptome RNA-seq of airway samples
  - This bulk RNA-seq will be performed on an aliquot of the specimens when thawed for scRNA-seq, to have matched bulk and single-cell data. Given the limited cell numbers we will perform bulk sequencing by a cell-direct library prep method. We recently optimized methods for generation of cell-direct libraries from <5,000 cells. We will apply these methods here.

- **Subtask 4.** Differential gene expression analysis of whole transcriptome
  - Not yet underway

- **Milestone(s) Achieved:** Single-cell and bulk RNA-seq and data analysis underway

- **Subtask 4.** Prepare and submit manuscripts for publication.
  - Not yet underway

Major Task 3: Determine the responses of lung resident immune cells to PM, type 2 inflammation (IL-12), and viral infection using human precision-cut lung slices (PCLS).

Major Task 3: Determine the responses of lung resident immune cells to PM, type 2 inflammation (IL-13), and viral infection using human precision-cut lung slices (PCLS)

- **Subtask 1.** Determine effect of PM, IL-13 and Influenza virus exposures on lung inflammation, injury, remodeling and airway hyperresponsiveness.
  - We have submitted the application to use cadaveric human explanted donor lung tissue has been submitted to OHRO
  - We have purchased a second Vibratome, set it up and tested it on human PCLS (funded by institutional funds and another grant)

- **Subtask 2.** Immune cell phenotyping and transcriptomic analysis in PCLS.

- Not yet underway

- **Subtask 3.** Mechanistic studies examining pathways discovered in Aims 1 and 2.
  - Not yet underway

- **Subtask 4.** Prepare and submit manuscripts for publication.
  - Not yet underway

	Year 1				Year 2				Year 3
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
Target Enrollment (per quarter)	0	3	6	6	6	6	6	6	0
Target Enrollment (cumulative)	0	3	9	15	21	27	33	39	60
Site 1 per quarter (Actual)	0	0	6						
Site 1 cumulative (Actual)	0	0	6						

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

An abstract reporting observations will be submitted to the American Thoracic Society annual International Conference in November 2022.

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?

In the next reporting period we plan to accomplish the following:
(i) Develop methods for mass cytrometry and single cell proteomic and immunophenotyping of lung innate immune cells.
(ii) Develop methods for analysis for respiratory epithelial resident innate immune cells.
(iii) Develop the human PCLS model to study innate immune cell function in response to *ex vivo* particulate matter exposure and influenza virus infection

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

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

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

We experienced delays in hiring personnel for the first 6 months of the grant because of COVID-19 related work force problems. We experienced delays in obtaining BAL fluid and cells from bronchoscopy specimens through the NJH Biobank and Honest Broker systems because of COVID-19 pandemic related restrictions

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

Nothing to report

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

Nothing to report

5. PRODUCTS:

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

Journal publications.

Nothing to report

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

Nothing to report

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

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Gregory P. Downey
Project Role: Principal Investigator
Researcher Identifier (ORCID ID):
Nearest Person Month Worked: 1.2 person months
Contribution to Project: Dr. Downey serves as the contact-PI and oversees and directs all aspects of the project. He meets with laboratory personnel on a daily basis and conducts weekly lab meetings. He is responsible for planning and coordinating the experiments, reviewing the data, preparing, and submitting progress reports to the DoD, attending the DoD progress meetings, and writing and submitting the manuscripts for publication.

Name: Hong Wei Chu
Project Role: Co-Investigator
Researcher Identifier (ORCID ID):
Nearest Person Month Worked: < 1 person month

Contribution to Project: Dr. Chu serves as Project Leader and directs the human precision cut lung slice models including influenza infection in the proposed studies. He coordinates the studies proposed in Aims 1 and 3. Dr. Chu is also the Director of the Mucosal Cell Core at NJH and will oversee culturing bronchial brushing cells. Dr. Chu will assist Drs. Rose, Day, Downey, and Seibold in data collection and manuscript preparation.

Name: Brian Day
Project Role: Co-Investigator
Researcher Identifier (ORCID ID):
Nearest Person Month Worked: < 1 person month
Contribution to Project: Dr. Day serves as a project leader and helps coordinate the studies proposed in Aims 1 and 3 including experiments using human precision cut lung slice models. He has bi-weekly laboratory meetings with the other investigators and their staff. Dr. Day reports the progress of the project to Dr. Downey and helps in data analysis, data reporting and manuscript preparation.

Name: Cecile Rose
Project Role: Co-Investigator
Researcher Identifier (ORCID ID):
Nearest Person Month Worked: < 1 person month
Contribution to Project: As Co-PI on this project, Dr. Rose is responsible for coordinating clinical research aspects of the grant scope of work including hiring and supervising the clinical Program/Research Coordinators; supervising the IRB submissions; overseeing the clinical database and data entry efforts; assuring that all clinical research samples are obtained and appropriately distributed to co-investigators as described; supervising research sputum induction; and assuring that results are successfully analyzed and published.

Name: Max A. Seibold
Project Role: Co-Investigator
Researcher Identifier (ORCID ID):
Nearest Person Month Worked: < 1 person month

Contribution to Project: Dr. Seibold serves as Project Leader and is responsible for the design and performance of the analyses in Specific Aim 2. Dr. Seibold participates in monthly meetings with all Co-Investigators and is available to provide advice and support for all areas of the project. He directly oversees the experiments proposed in Specific Aim 2 and the analysis of RNA-seq and genomic data for Specific Aim 3.

Name: Matthew Strand
Project Role: Co-Investigator
Researcher Identifier (ORCID ID):
Nearest Person Month Worked: < 1 person month
Contribution to Project: Dr. Strand serves as the lead biostatistician and is responsible for power calculations for experiments, the design and performance of the analyses of data, particularly complex nonparametric analyses and linear and not-linear modeling. Dr. Strand participates in monthly meetings with all Co-Investigators and is available to provide statistical advice and support for all areas of the project.

Name: Kelly Correll
Project Role: Senior Researcher
Researcher Identifier (ORCID ID):
Nearest Person Month Worked: 3.3 person months
Contribution to Project: Ms. Correll is responsible for coordinating the receipt of the donor lungs and all additional preparations required for the human lung epithelial cell isolations and for human precision cut lung slices. Ms. Correll is responsible for all of the immunohistochemistry and immunofluorescence microscopy.

Name: Katrina Diener
Project Role: Senior Researcher
Researcher Identifier (ORCID ID):
Nearest Person Month Worked: 1.5 person months
Contribution to Project:

Name: Jamie Everman
Project Role: Senior Researcher
Researcher Identifier (ORCID ID):
Nearest Person Month Worked: 1.1 person months
Contribution to Project: Dr. Everman works under the direction of Drs. Seibold and Chu and is responsible for generation of all airway epithelial cultures and precision cut lung slices, and the generation of RNA-seq libraries.

Name:	Elysia Min Hawkins
Project Role:	Senior Researcher
Researcher Identifier (ORCID ID):	
Nearest Person Month Worked:	1.9 person months
Contribution to Project:	Ms. Min participates in the studies outlined in Aims 1 and 2 including the precision cut lung slices and mass cytometry studies.

Name:	Kath Pang
Project Role:	Clinical Research Coordinator
Researcher Identifier (ORCID ID):	
Nearest Person Month Worked:	<1 person months
Contribution to Project:	MS. Pang assists with study subject recruitment and informed consenting; clinical research data collection (including blood draws and questionnaire administration); data entry and data quality assurance; assistance with obtaining, cataloguing, and handling nasal and bronchial brushings, and bronchoalveolar lavage fluid and cells; and other aspects of project coordination as needed and with oversight from Dr. Rose and the Project Coordinator.

Name:	Satria Sajuthi
Project Role:	Biostatistician
Researcher Identifier (ORCID ID):	
Nearest Person Month Worked:	1.7 person months
Contribution to Project:	

Name:	Niccolette Schaunaman
Project Role:	Senior Researcher
Researcher Identifier (ORCID ID):	
Nearest Person Month Worked:	1.8 person months
Contribution to Project:	Ms. Schaunaman is responsible for assisting with the precision cut lung slices and nasal and airway epithelial culture.

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

Since the award of this project, the following changes to active other support have occurred.

- Dr. Gregory P. Downey completed work on NIH projects, R01HL132950 entitled, "PTPα as a Checkpoint in Fibrogenic Responses in the Lung" and U01HL131755 entitled, "Multicenter Interventional Lymphangioleiomyomatosis Early Disease Trial (MILED)-CCC". He completed work on DOD Focused Project award, W81XWH-16-2-0018 entitled, Mechanisms and Treatment of Deployment-Related Lung Injury: Repair of the Injured Epithelium". Dr. Downey was awarded NIH project, R01HL157424 entitled, "Proteolysis in the Pathogenesis of ARDS". In addition, Dr. Downey was awarded a Savara Inc. project, SAV006-5 entitled, "A randomized, double-blind, placebo-controlled clinical trial of once-daily inhaled molgramostim nebulizer solution in adult subjects with autoimmune pulmonary alveolar proteinosis (aPAP) (IMPALA-2)". Dr. Downey began work on the American Lung Association project, COVID-ETRA 736704 entitled, "Novel risk factors for severe COVID-19 lung disease". The addition of these projects will have no impact on his effort on this project.

- Dr. Cecile Rose completed work on the DOD Focused Project award, W81XWH-16-2-0018 entitled, Mechanisms and Treatment of Deployment-Related Lung Injury: Repair of the Injured Epithelium".

- Dr. Hong Wei Chu completed work on the DOD Focused Project award, W81XWH-16-2-0018 entitled, "Mechanisms and Treatment of Deployment-Related Lung Injury: Repair of the Injured Epithelium". He completed work on the NIH projects U19AI125357 (year 1 to year 5) entitled, "Dysfunction of Innate Immunity in Asthma: Role of Tollip in dysfunction of asthma airway innate immunity" and R01HL135156 entitled, "Transcriptomic and Pharmacogenetic Asthma Endotypes in Minority Children". Dr. Chu completed work on the American Lung Association project, ETRA 736704 entitled, "Novel risk factors for severe COVID-19 lung disease". Dr. Chu began work on the NIH projects R01AI161296 entitled, "Novel Biased Beta2-AR Ligands as Asthma Therapeutics", R01HL157424 entitled, "Proteolysis in the Pathogeneses of ARDS", and R01HL109557 entitled, "Mechanisms and Consequences of Gene Induction by Glucocorticoids in Airway Smooth Muscle". The addition of these projects will have no impact on his effort on this project.

- Dr. Brian Day completed work on the DOD Focused Program award, W81XW-16-2-0018 entitled, "Mechanisms and Treatment of Deployment-Related Lung Injury: Repair of the Injured Epithelium", and on the Cystic Fibrosis Foundation project, DAY18GO entitled, "Manganese porphyrin selenocyanates as a novel antimicrobial therapy against CF pathogens". Dr. Day was

awarded NIH Project R21ES032951 entitled, "Repurposing thiosulfate for chlorine gas exposure". The addition of this project will have no impact on his effort on this project.

- Dr. Max Seibold completed work on NIH projects, R01HL128439 entitled, "Genetic Control of Airway Epithelium Gene Expression in Childhood Asthmatics", R01AR041256 entitled, "The Role of Bacterial Toxins in Human Skin Disease", and R01HL140595 entitled, Fibroblast Resistance to Apoptosis in Pulmonary Fibrosis". He completed work on the NIH supplement, UM1AI151958-01S1 entitled, "Experimental and Computational Analysis of the Human Epidemiology and Response to SARS-CoV-2 (HEROS) Cohort". Dr. Seibold completed work on Genentech Inc. project, CRISPER and the DOD Focused Project award, W81XWH-16-2-0018 entitled, "Mechanisms and Treatment of Deployment-Related Lung Injury: Repair of the Injured Epithelium". Dr. Seibold began work on NIH supplements, UM1AI151958-02S1 entitled, "Airway transcriptomic responses to COVID-19 Illnesses in the Human Epidemiology and Response to SARS-CoV-2 (HEROS) Cohort" and UM1AI151958-03S1 entitled, "Deciphering heterogeneity of airway molecular response and risk for SARS-CoV-2 infection in the Human Epidemiology and Response to SARS-CoV-2 (HEROS) Cohort. Dr. Seibold began work on NIH project, U01AI160033 entitled, "Critical Windows in the Development of Asthma Endotypes and Phenotypes in High-Risk Toddlers". Dr. Seibold serves as mentor on NIH project, K23HL161354 entitled, "Novel Markers of Treatment Responsiveness for Pediatric Acute Asthma Exacerbations". Dr. Seibold will begin work on NIH project R01HL080414 (years 13-17) entitled, "Phenotypic and biological features of mucus plug in asthma" as soon as the IRB protocol is approved at National Jewish Health. The addition of these projects will have no impact on his effort on this project.

- Dr. Matthew Strand completed work on DOD Focused Project award, W81XWH-16-2-0018 entitled, "Mechanisms and Treatment of Deployment-Related Lung Injury: Repair of the Injured Epithelium". He completed work on NIH award R01HL130559A entitled, "Stepped-care Management of Insomnia Co-occurring with Sleep Apnea" and the Cystic Fibrosis Foundation award TAYLOR19AO entitled, "Impact of triple combination CFTR therapy on sinus disease". Dr. Strand was awarded a State of Colorado award CTGG120222905Strand entitled, "Software Development for COPD Prediction Models". He began work on the Cystic Fibrosis Foundation project HARRIS19A0 entitled, "Mechanisms of Exercise Intolerance in Cystic Fibrosis: Role of PDE5 Inhibition". The addition of these projects will have no impact on his effort on this project.

What other organizations were involved as partners?

Drs. Karen Mumy and Brian Wong (retired) from NAMRU-D supplied us with aerosolized and size fractionated particulate matter from Afghanistan. This was prepared from top soil collected near Bagram Air Force Base aerosolized using a Wright Dust Feeder particle generator and size fractionated.

6. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

7. APPENDICES: