Epithelial Repair Dysfunction in the Pathogenesis of Deployment-Related Lung Disorders

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Disease Heterogeneity: A Major Issue for Diagnosis

PFT is largely normal with the exception of Low Diffusion Capacity (DLCO)

Epithelial Repair a Key Determinant of Lung Health

Epithelial Stem/Progenitor Cells

Replenish themselves (Self-renewal)

Make all cell types of the airways (Multipotentiality)

Stem/Progenitor Cell
Progenitor Dysfunction in COPD

Ghosh M, et al. AJRCCM, 2018
Progenitor Dysfunction in Premalignant Lung 

Bronchial Premalignancy: Squamous cell cancer

Lung organoid

Distal lung Premalignancy: Adenocarcinoma

Progenitor cell clone

Log Progenitor Self-renewal at BL

p = 2.8 e-08

p = 1.4 e-06

p = 0.0015

Number of organoids (per 10^6 epithelial cells)
Global Dysfunction of Epithelial Progenitor Cells and Loss of Epithelial Repair Contributes To the Development of Smoking Related Lung Disease
Chronic Inflammation in Pathogenesis of DDLD

- Cell differentials in bronchoalveolar lavage — Variable
- Acute Eosinophilic Inflammation — Variable
- Mouse models exposed to desert particulate from Afghanistan (APM) — Neutrophilic inflammation, activated monocytes
- In vitro human airway culture exposed to APM — Type 2 immune response
Global Epithelial Dysfunction and Inflammation Contributes to DDLD Pathogenesis

Deployment Related Exposure

Central airway progenitor dysfunction and underlying molecular mechanisms

Aim 1

Deployment Related Distal Lung Disease (DDLD)

Lung epithelial inflammation

Aim 3

Distal airway progenitor dysfunction and underlying molecular mechanisms

Aim 2

Aim 3

Aim 1
Research Goals

Deployment-related Exposure

**Inclusion:** Age: 25-45 yrs
Smoking history: < 10 pack-years

**Exclusion:** Pre-existing respiratory symptoms
Chronic lung or cardio vascular disease
Sarcoidosis

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Deployers diagnosed with DDLD (Group 1)

N = 50

i) Clinical Assessment/Administration of respiratory health related questionnaire
ii) Peripheral brushings with fluoroscopically-guided bronchoscopy
iii) Endobronchial biopsy
iv) Bronchoalveolar lavage

Follow-up

VA Longitudinal Evaluation and Assessment Program (LEAP)

Deployers without symptoms with normal PFT and HRCT (Group 2)

N = 50

i) Clinical Assessment/Administration of respiratory health related questionnaire
ii) Peripheral brushings with fluoroscopically-guided bronchoscopy
iii) Endobronchial biopsy
iv) Bronchoalveolar lavage

Follow-up

VA Longitudinal Evaluation and Assessment Program (LEAP)

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Research Team

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CU - Core Facilities
Clinical Trial Core
Bioinformatics and Biostatistics Core
Genomics and Microarray Core
Human Immune Monitoring
Shared Resources
Flow Cytometry Shared Resources
Current Clinical Research Studies in Post-Deployment Respiratory Health at VA ECHCS

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Opinions, interpretations, conclusions and recommendations are my own and are not necessarily endorsed by the Department of Veterans Affairs or the Department of Defense.
COMIRB 19-1853: Clinical Markers and Monitoring for Post-9/11 Deployment Lung Diseases
Longitudinal Evaluation and Assessment Program (LEAP) Study (PI: Krefft)

Overall Goal: development of a well-characterized cohort of Southwest Asia deployed veterans with and without lung disease

SPECIFIC AIMS:

• **Aim 1**: Aim 1a - Recruit and characterize a VA cohort of post-9/11 southwest Asia and Afghanistan deployment (SWAAD) veterans with and without deployment-related respiratory symptoms (DRS)/deployment-related lung disease (DLD).

• **Aim 2**: Characterize peripheral blood samples using Cytometry by Time-of-Flight (CyTOF) to identify cellular biomarkers of DLD and determine if there are differences in inflammation in a cohort of veterans with and without post-9/11 SWAAD DRS/DLD.

• **Aim 3**: Compare *longitudinal respiratory outcomes* in the established VA cohort of post-9/11 southwest Asia and Afghanistan deployment veterans with and without DRS/DLD.
**Study Protocol**

**CONTROLS** – from VA Airborne Hazards and Open Burn Pit Registry Evaluation Clinic

- Deployers without cough, wheezing, chest tightness, dyspnea *(CONTROLS)*

  - COMPLETE PERIPHERAL BLOOD DRAW, BASELINE
  - SPIROMETRY (+DLCO in smokers), LCI TESTING, AND modified QUESTIONNAIRE
  - Repeat spirometry (+DLCO in smokers), LCI TESTING and QUESTIONNAIRE at a visit 15-18 months later *(n = 55)*

**EXCLUDED IF:**
- Alternate diagnosis (e.g. pre-deployment asthma, sarcoidosis)
- Lung cancer
- Cardiomyopathy

**Recruited from VA Chest Clinic, Chest Exposure Clinic, and Specialty Post-Deployment Cardiopulmonary Evaluation Network Clinic**

- Deployers with respiratory symptoms (includes those with DRA and DDLD)

  - COMPLETE PERIPHERAL BLOOD DRAW, BASELINE
  - PFT, LCI TESTING, AND QUESTIONNAIRE
  - Repeat PFT or spirometry/DLCO, LCI testing and QUESTIONNAIRE at a visit 15-18 months later *(n = 150)*

**Progress to Date:**
- Consented/enrolled 182 symptomatic deployers, 56 controls
- 165 completed baseline study visits
- 36 completed follow-up study visits – completed study
COMIRB 21-3104: VA Gulf War Era and Post-9/11 Southwest Asia & Afghanistan Deployment (SWAAD) Research Database and Biorepository

- Recruitment populations:
  - VA patients who served in southwest Asia (particularly those who deployed during the First Gulf War from 1990-1991 and in more recent military operations in southwest Asia and Afghanistan in the post-9/11/2001 era) as well as during peacetime operations

- Biorepository and database
  - Collect data via medical chart review and completion of questionnaires on deployment, military and medical history (REDCap database)
  - Peripheral blood sample to be banked for future research
  - Enrolling study participants currently, symptomatic and healthy veterans

- PI: Silpa Krefft, Co-Investigators: Arnav Gupta, Alison Wilczynski
- Number of study participants: Up to 5000 eligible veterans

Funded by Department of Veterans Affairs
COMIRB 21-2804: Study to Improve Deployment Related Asthma by Using L-Citrulline Supplementation (SEALS)
PIs: Fernando Holguin (CU), Cecile Rose (NJH); Silpa Krefft (VA Site Leader)

• Substantial number of veterans with deployment-related asthma (DRA) have non-Th2 (or Th2)-low asthma endotype.
• Novel therapies are needed for non-Th2 asthma.

• Clinical trial (multi-site at CU/NJH/VA) with target enrollment of 75 study participants (veterans with DRA from First Gulf War era through the post-9/11 era) over the next 4 years
  • Actively recruiting/enrolling now

• Project Aims of phase II proof of concept study are to demonstrate the efficacy and safety of L-citrulline supplementation, in addition to asthma controller medications, for improving the following in those with deployment-related asthma (DRA):
  • Asthma control
  • FeNO
  • Lung function

Funded by Department of Defense
COMIRB 22-2069: High-Intensity Interval Training (HIIT) to Improve the Symptoms of Deployment-Related Respiratory Disease (DRRD)

• ~50% of participants with DRRD (asthma and/or distal lung disease/small airways disease such as bronchiolitis) demonstrate reduced VO2max

• In this pilot study, we will study 12 veterans with DRRD
  • Echocardiography, venous metabolomics, and cardiopulmonary exercise testing (CPET) before and after a 12-week high-intensity interval training exercise intervention
  • Generate pilot data regarding impact of HIIT on cardiopulmonary performance and its efficacy in improving functional capacity and symptoms in deployment-related respiratory disease

• Patient enrollment: May 2023 – October 2023

• PI: Lindsay “Shelley” Forbes, MD

• Co-Investigators: William Cornwell, MD, MSCS; Silpa Krefft, MD, MPH

• Funded by: VA Airborne Hazards and Burn Pit Center of Excellence (AHBPCE) Pilot Project Research Award FY2023-001
Background: Particulate exposures may cause DNA damage through generation of reactive oxygen species, and increased mtDNA injury has been associated with mitochondrial dysfunction.

Objective: Conduct a more detailed investigation into mitochondrial function and mtDNA lesions in PBMCs from Veterans with deployment-related lung disease (DLD).

Aim 1: Identify changes in the mitochondrial oxygen consumption rate in PBMCs obtained from patients with deployment-related lung disease (DLD) that includes asthma and deployment-related distal lung disease.

• Hypothesis: Mitochondrial dysfunction, evidenced by a decrease in mitochondrial respiration, is a pathologic feature of PBMCs obtained from patients with DLD.

• Approach: Measure extracellular acidification rate and oxygen consumption rate using the Agilent Seahorse XF assay in PBMCs obtained from 10 veterans with DLD and 10 deployed healthy controls.

Aim 2: Demonstrate an increased burden of mtDNA lesions and increased mtDNA copy number in PBMCs obtained from patients with DLD.

• Hypothesis: mtDNA from patients with DLD will exhibit a greater burden of mutations and per-cell copy number compared with controls.

• Approach: Quantify mtDNA lesions and copy number per cell using quantitative PCR-based assays in 10 veterans with DLD and 10 controls.

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