



Respiratory Consequences of Exposure to Airborne Particulate Matter

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Disclosures

- **Gregory Downey MD has no conflicts to report**
- **Brian Day Ph.D. has no conflicts to report**
- **Hong Wei Chu M.D. has no conflicts to report**
- **Max Seibold Ph.D. has no conflicts to report**



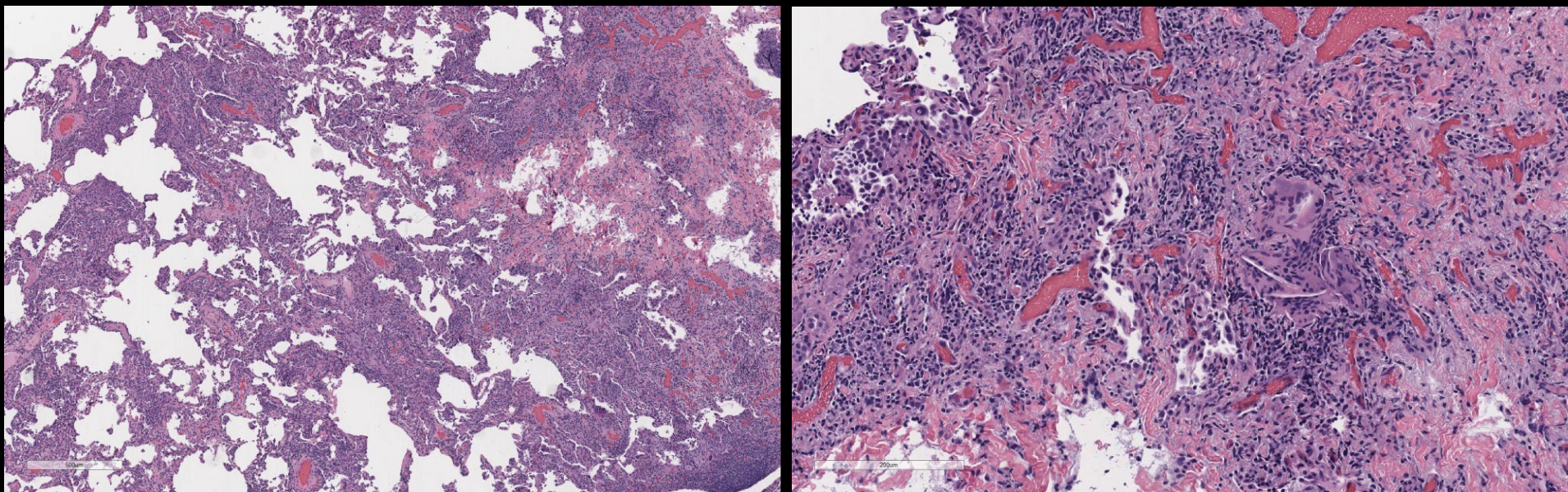
Dust Storm Approaching Military Camp in Iraq



Deployment Lung Disease

- >3 million military personnel in Southwest Asia and Afghanistan
- Exposed to high levels of dust (particulate matter – PM)
 - Generated by wind erosion of desert sand, movement of vehicles and troops, combustion and construction activities, and explosions from IEDs
 - Levels of ambient PM >10,000 $\mu\text{g}/\text{m}^3$ (10 mg/m^3) for days is common
 - Far higher than the WHO air quality guideline of 20 $\mu\text{g}/\text{m}^3$
- Exposed to indoor and outdoor aeroallergens (molds, date palm pollens) and airborne products of burned trash (burn pits)
- Increase in respiratory symptoms, asthma, bronchiolitis, eosinophilic/interstitial lung disease

Bronchiolitis and ILD in Soldier Deployed to Iraq and Afghanistan



- H& E stained sections from a VATS lung biopsy of a 58 year-old soldier referred for “intractable asthma” following deployment to Iraq and Afghanistan
- Biopsy reveals evidence of bronchiolitis with inflammation and fibrosis around the terminal airways extending into to the alveolar ducts and interstitium with presence of scattered granulomas

Goals

- Determine the effects of respiratory exposure to PM from Southwest Asia and Afghanistan in preclinical (mouse) models
- Determine the transcriptional effects on primary human cells of exposure to PM from Southwest Asia and Afghanistan
 - Primary human lung airway epithelial cells
 - Primary human alveolar macrophages

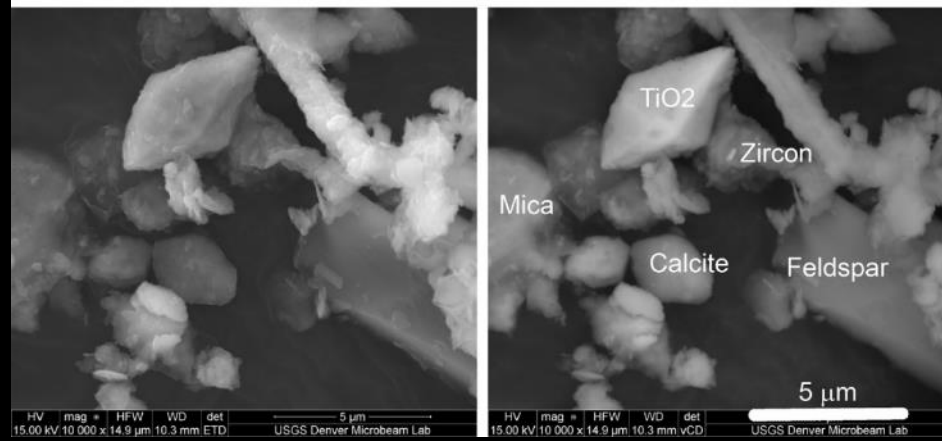
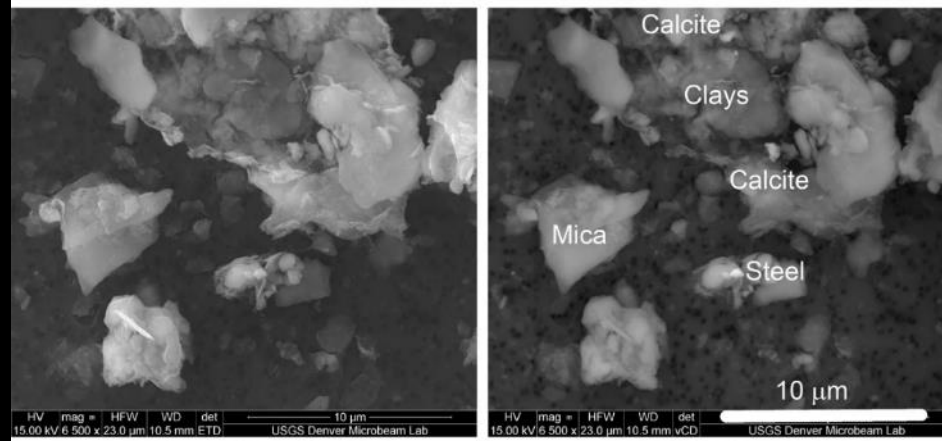
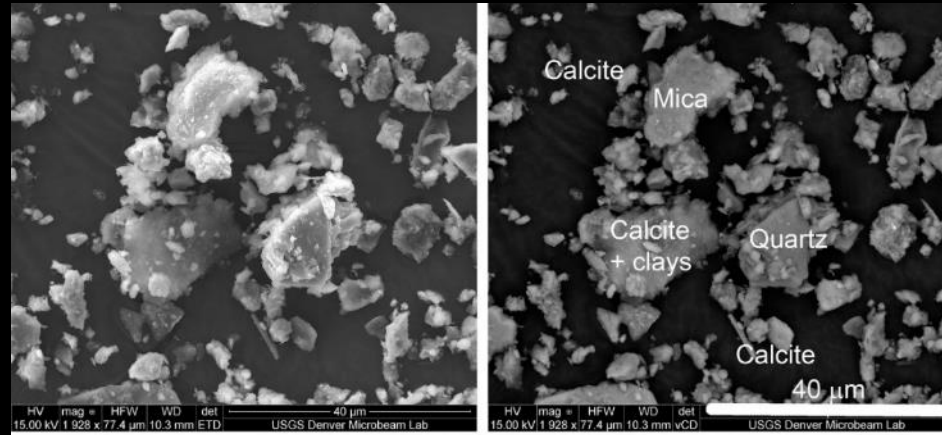
Animal Models of Respiratory PM Exposure

- C57Bl/6J mice age 8-12 weeks
 - Male and female
- **Single dose PM** → 5-25 mg/kg given by oropharyngeal aspiration
 - PM from Iraq, Afghanistan, or China Lake (California)
- **Multiple dose PM** → 5 doses (2.5 and 5 mg/kg) given by oropharyngeal aspiration every second day for 10 days

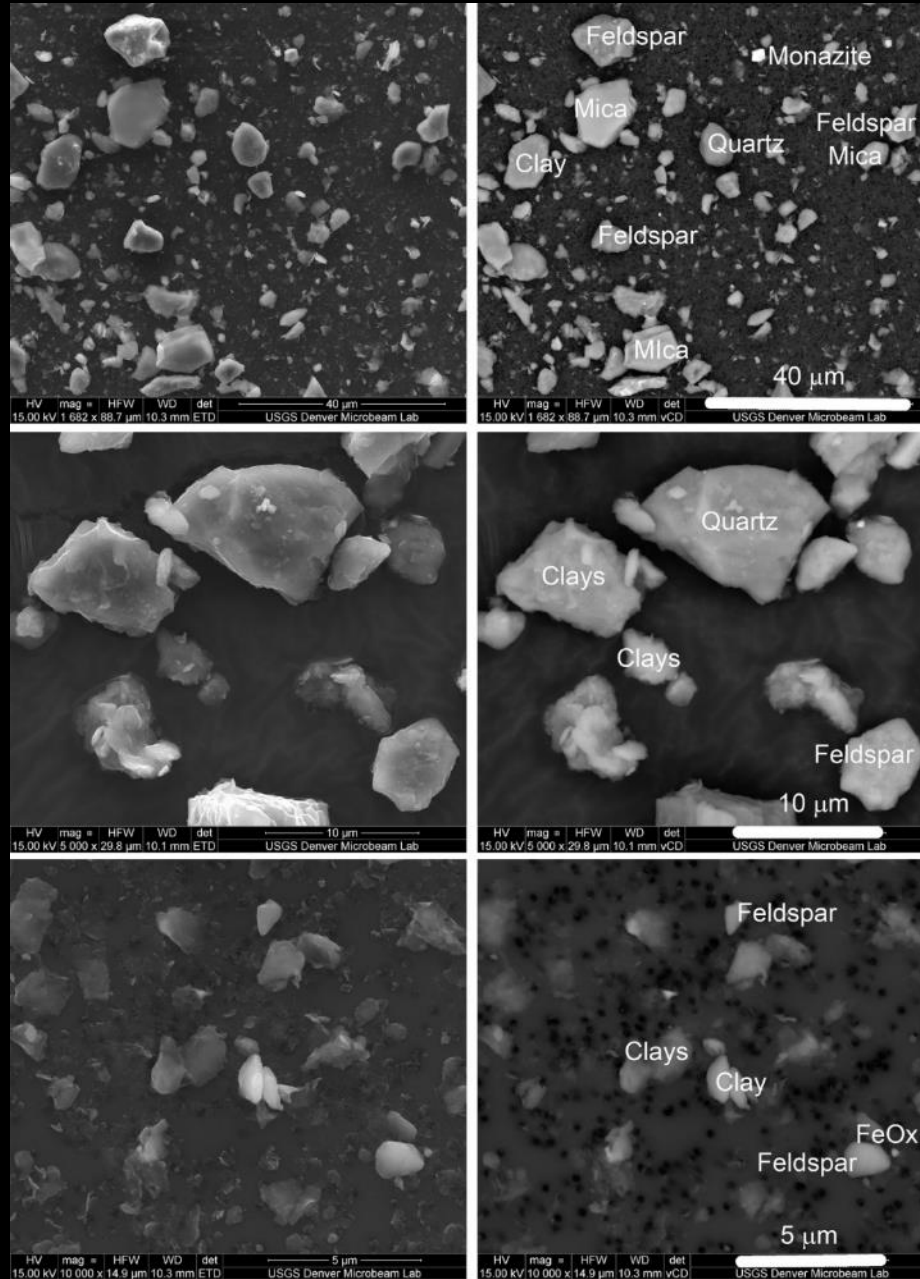
Characterization of Particulate Matter

- Particulate matter (PM) from Southwest Asia and North America
- **Iraq** (Camp Victory - Near Bagdad; courtesy of Dr. David Jackson)
 - Ambient PM collected on high volume Teflon filters
 - Sterilized by γ -irradiation
 - Median size 5.3 μm
 - Chemical and elemental analysis published (Desert Research Institute)
 - SiO_2 38.9%; Al_2O_3 9.6%; Fe_2O_3 5.3%; TiO_2 0.6%)
- **Afghanistan** (Bagram Airfield 60 km from Kabul)
 - Sand/topsoil collected in large drums
 - Sterilized by autoclaving
 - Sifted and then aerosolized and size fractionated at NAMRU-Dayton (median size 5.1 μm)
 - Collected on Teflon filters
- **California** (China Lake/ Fort Irwin)
 - Sifted – then aerosolized and size fractionated (median size 5.2 μm)
 - Collected on Teflon filters
- **Purified silica** used for comparison

Afghanistan Particulate Matter

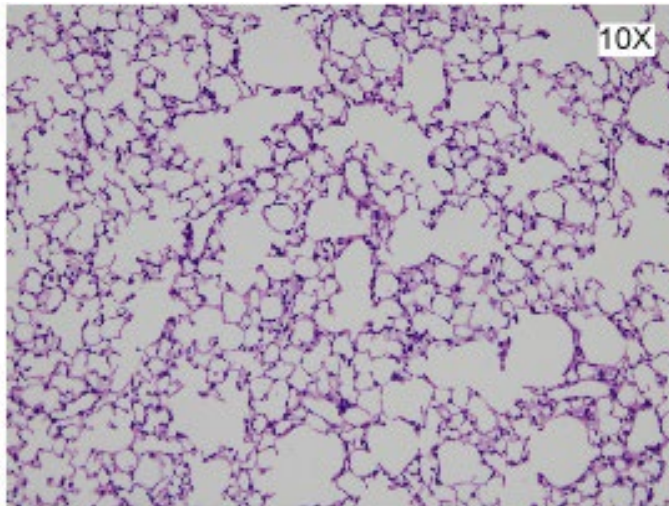


China Lake Particulate Matter

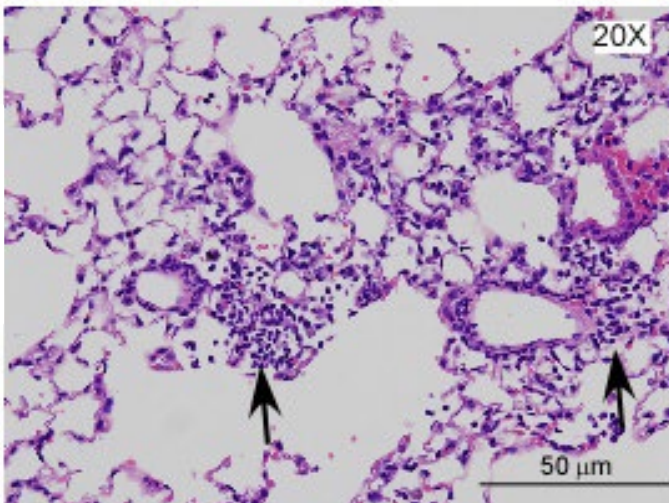
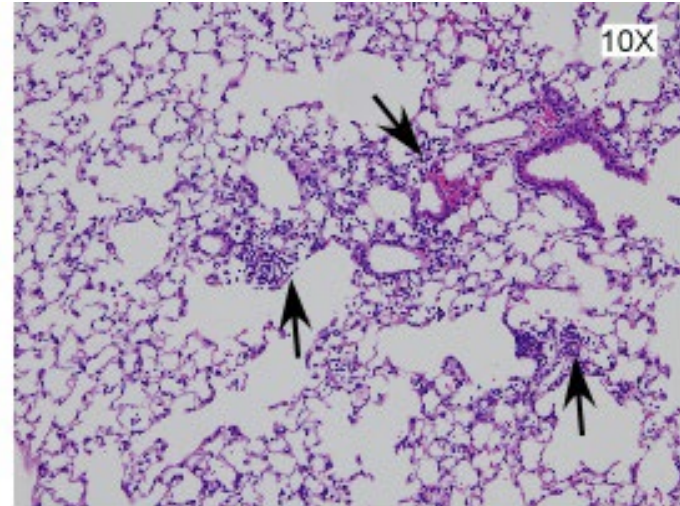


Intratracheal Instillation of PM from Southwest Asia Induces Inflammation Around the Terminal Bronchioles and Alveolar Ducts

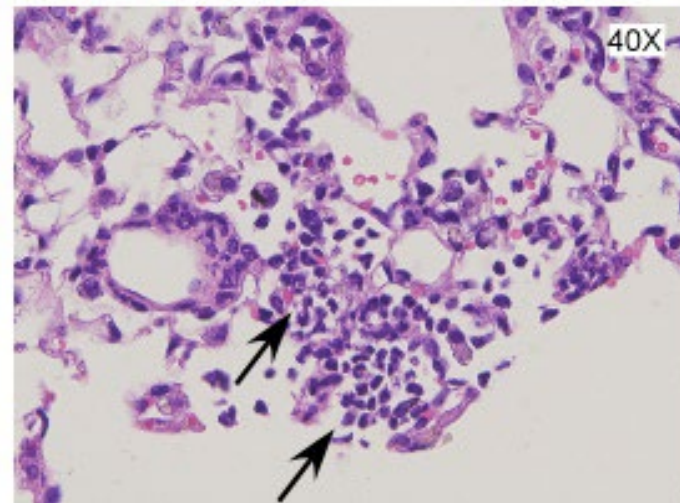
Saline Control



SWA PM 24 hr



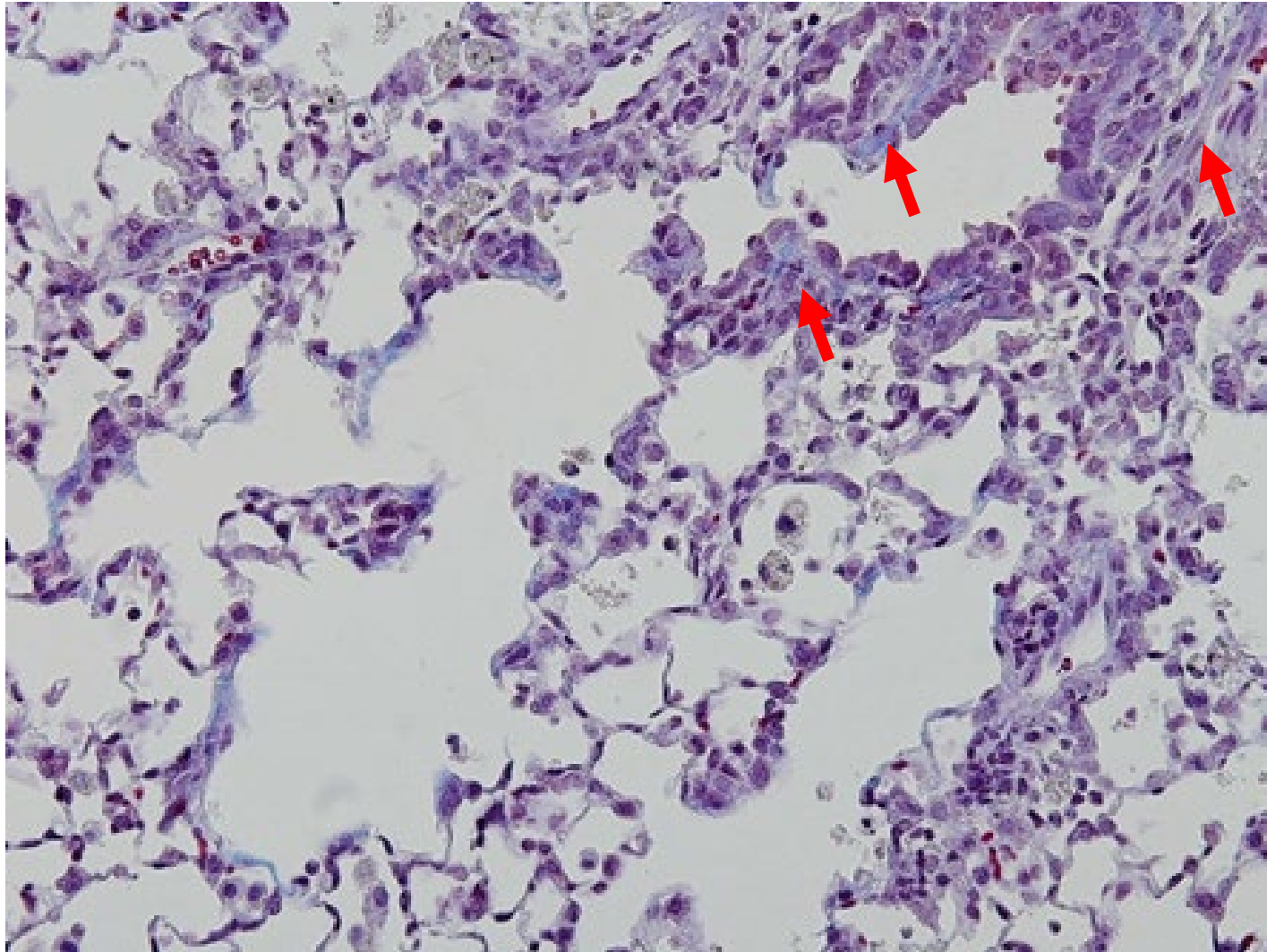
SWA PM 24 hr



SWA PM 24 hr

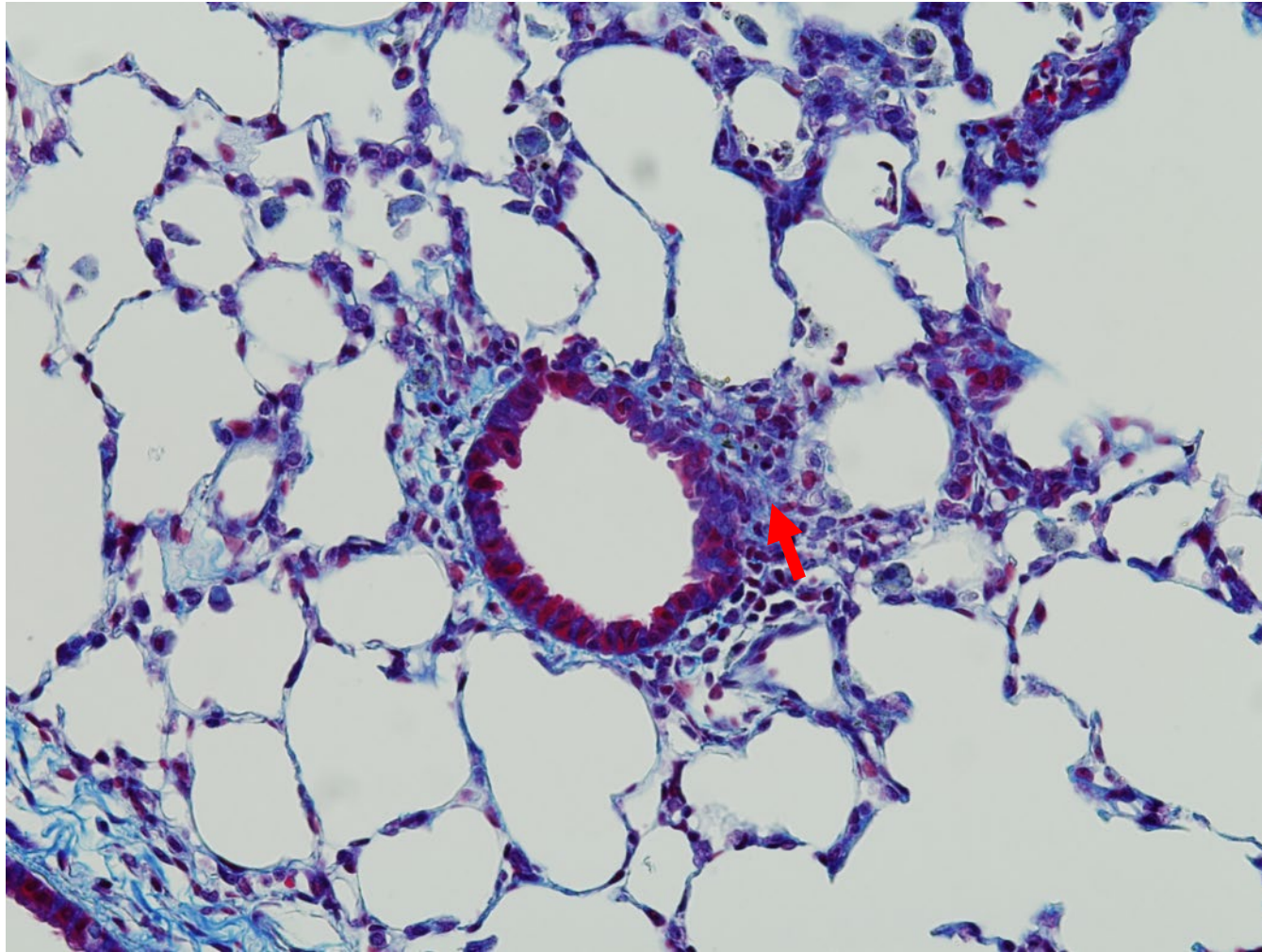
H&E stain

Instillation of PM from Southwest Asia Induces an Increase in Lung Collagen Content



Repetitive instillation model: 5 doses over 10 days
Masson's Trichrome Stain – collagen stains blue

Repetitive (5 doses) Instillation of PM from Southwest Asia Induces Inflammation and Peribronchiole Fibrosis

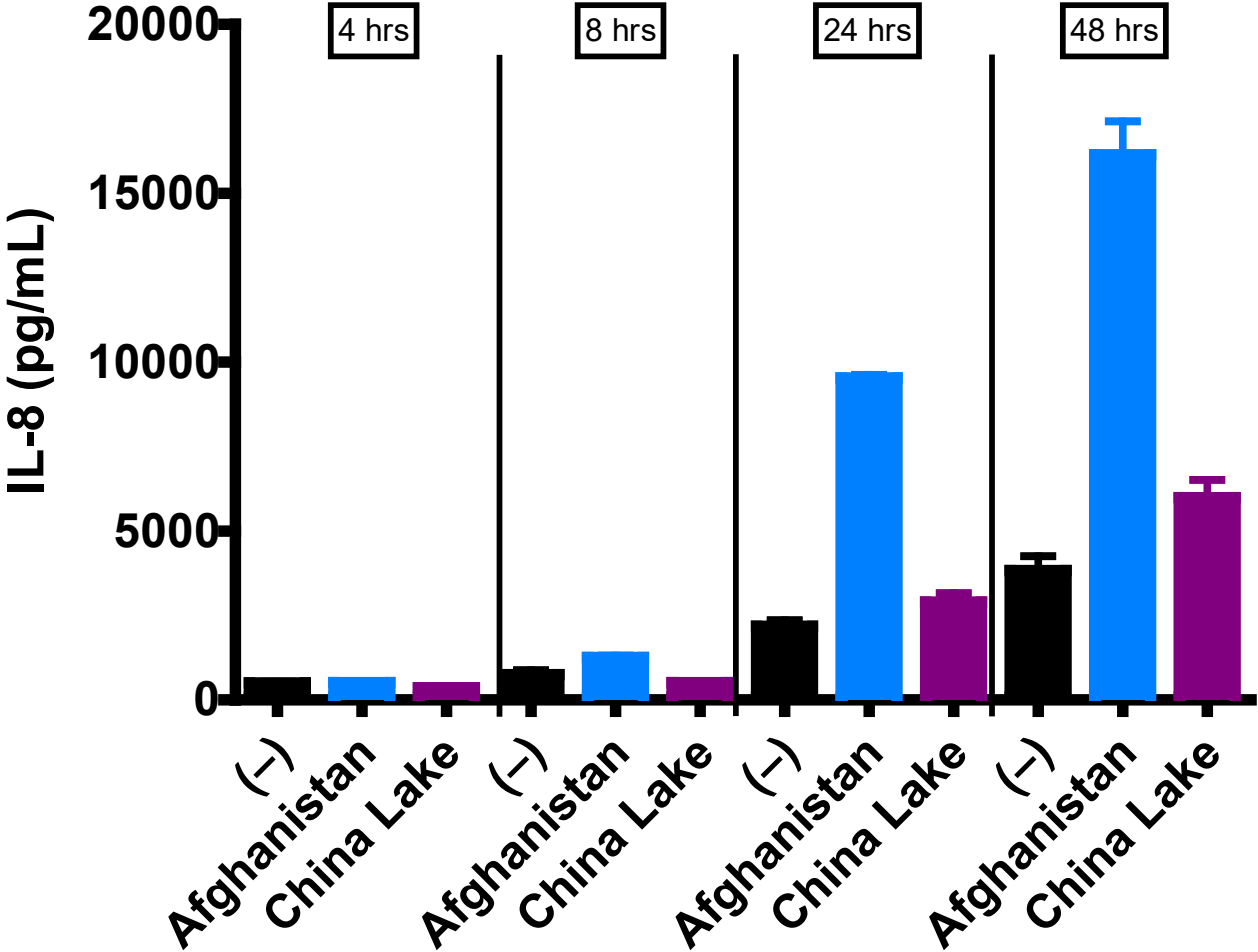


Masson's Trichrome staining of lung sections (20X)

Effects of PM Exposure on Primary Human Airway Epithelial Cells

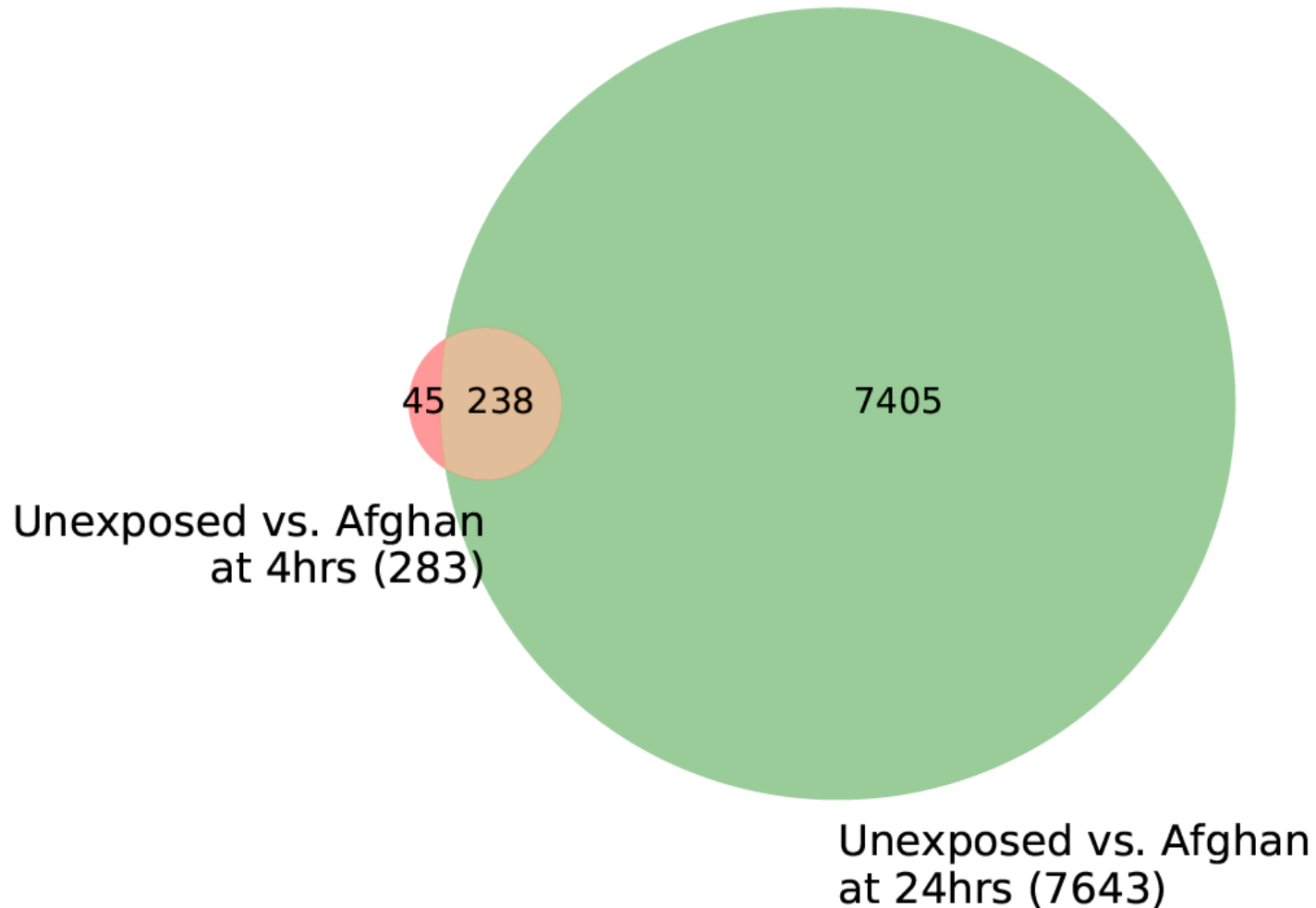
Exposure of Primary Human Airway Epithelial Cells to PM from Afghanistan Induces IL-8 Expression

Time Course



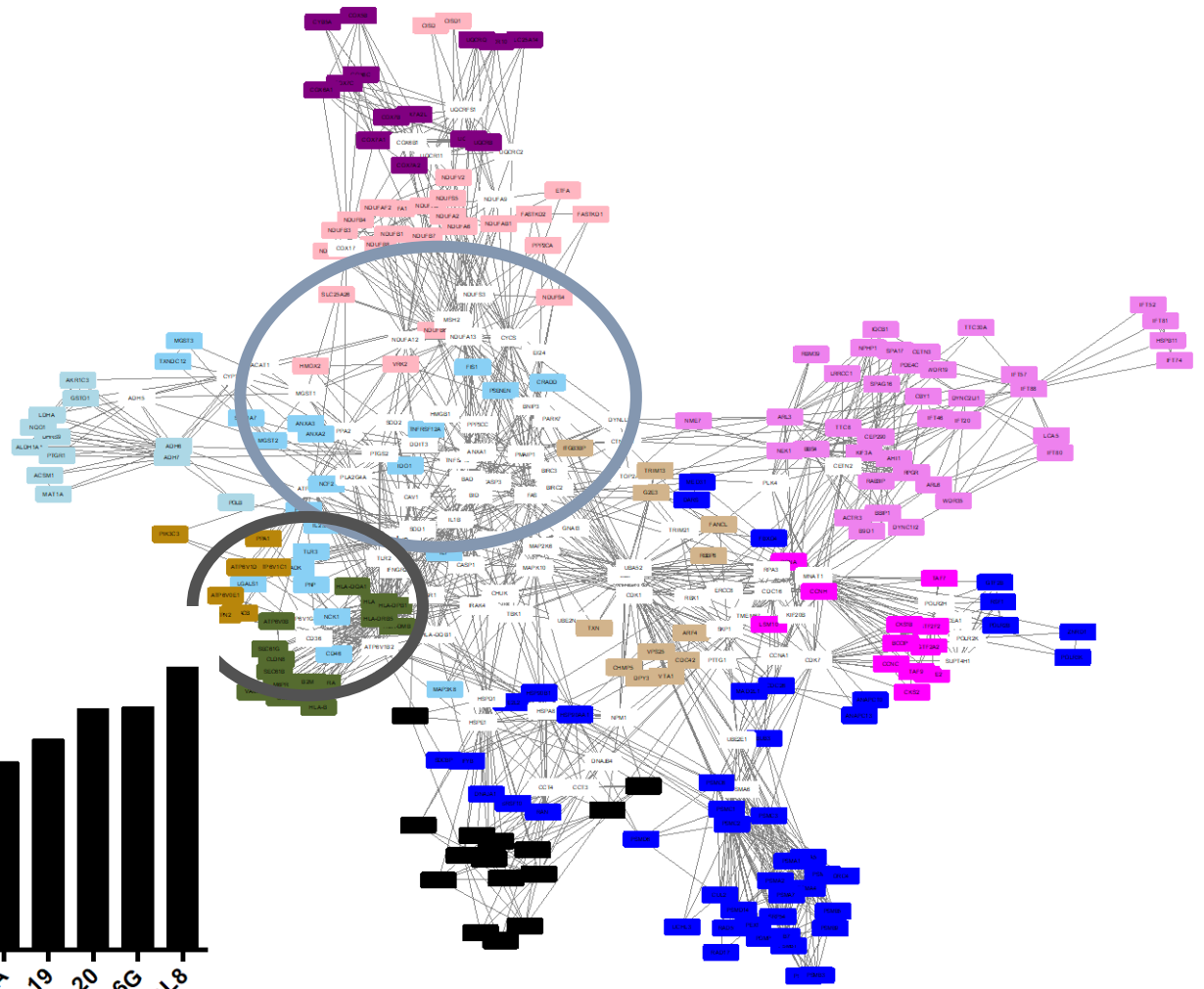
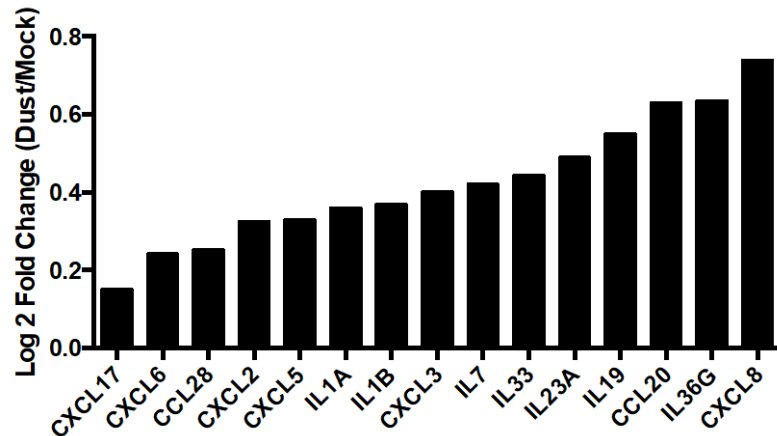
RNA-Seq Analysis of Differentially Expressed Genes (DEG) in Response to PM Exposure

Commonalities among Unexposed vs. Afghan DEGs at 4 and 24 hours (FDR < 0.05)



Functional enrichment analysis of epithelial response to Iraq PM

Immune response
Cytokine response
Antigen presentation
Immune response
Cytokine-cytokine receptor
Response to LPS/bacteria



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Immune Cell Enrichments

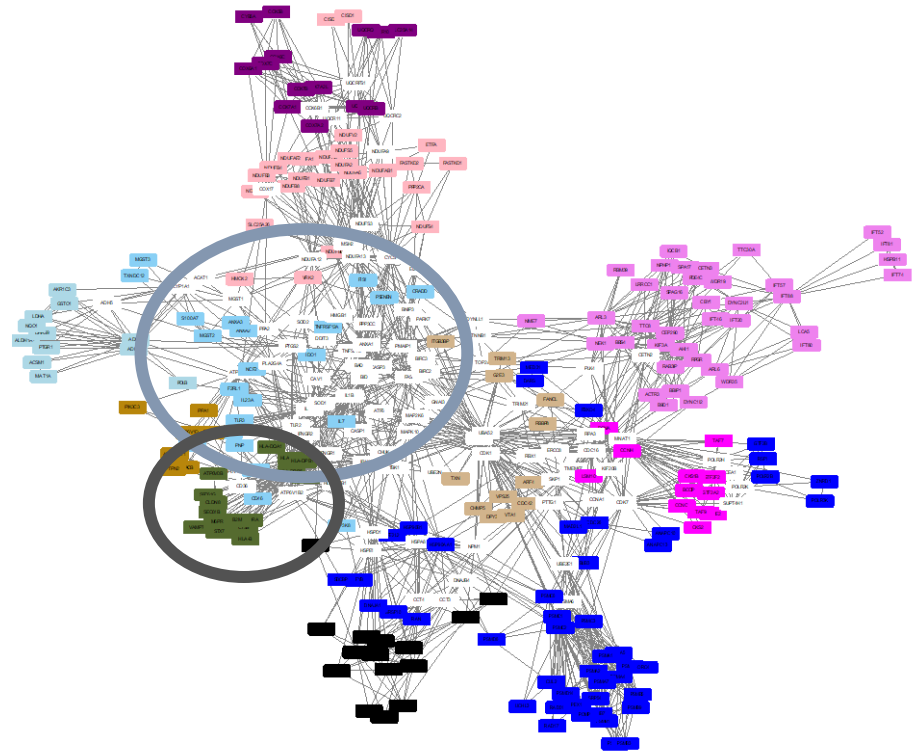
Neutrophil Chemotaxis

Monocyte Recruitment

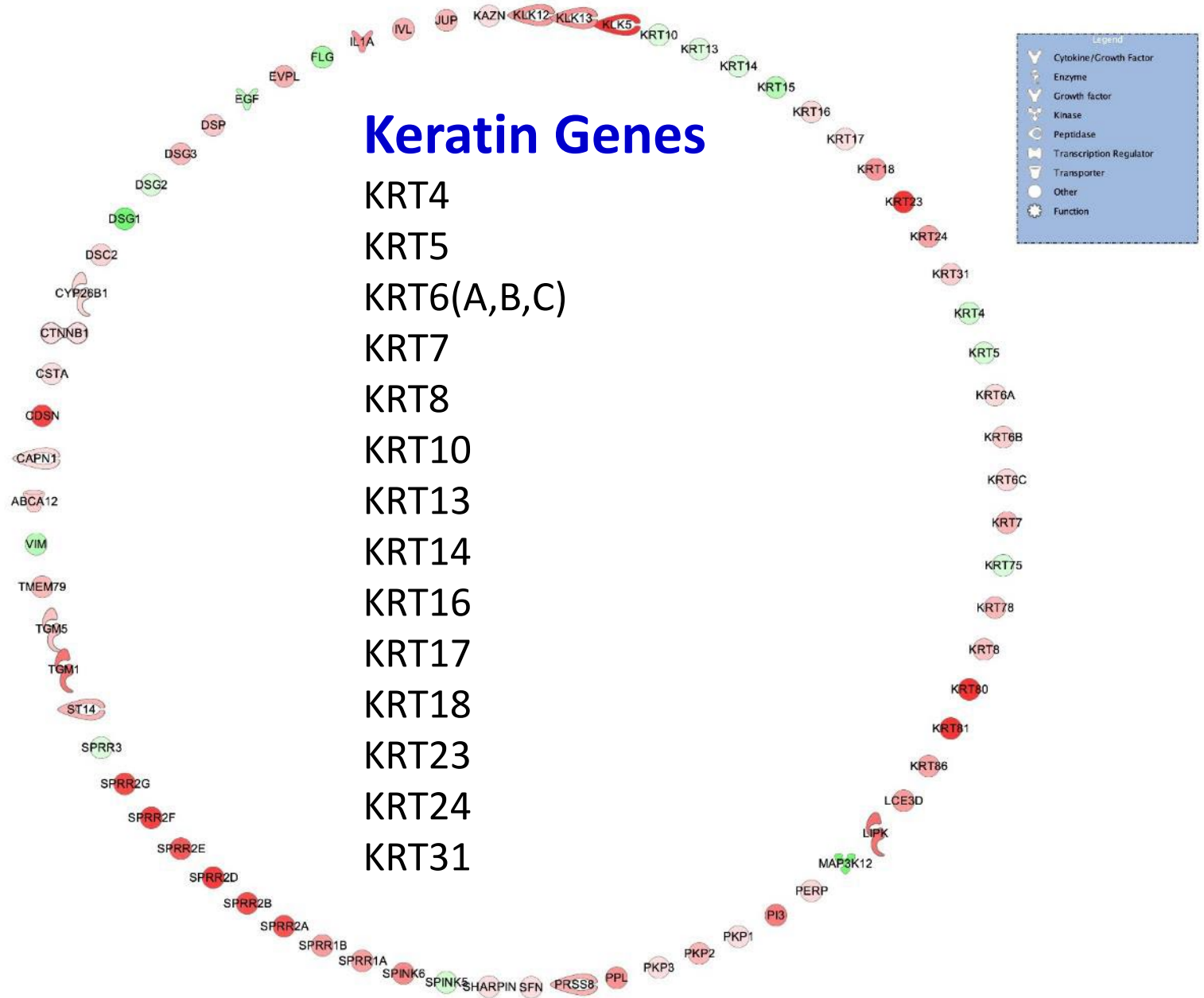
Macrophage Chemotaxis

Leukocyte Recruitment

Granulocyte Chemotaxis



Keratinization Pathway

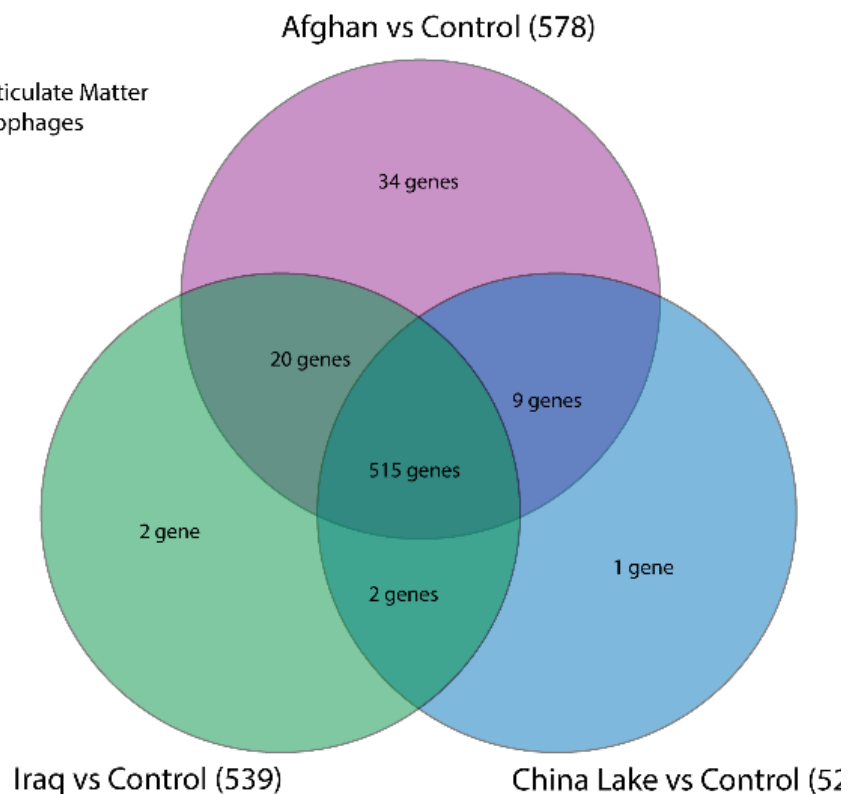


Effects of PM Exposure on Primary Human Alveolar Macrophages

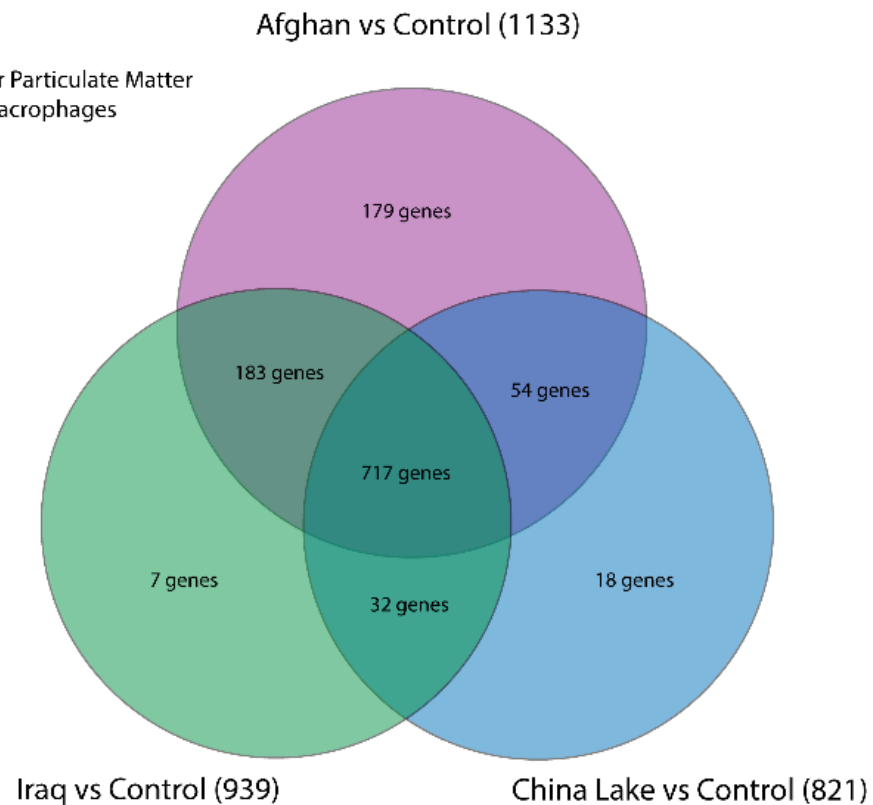


Exposure to PM from Southwest Asia/Afghanistan Induces Differential Gene Expression in Primary Human Alveolar Macrophages

4 hr Particulate Matter in Macrophages



24 hr Particulate Matter in Macrophages



Conclusions

- PM exposure in mice induces early neutrophilic inflammation and late peribronchiolar fibrosis
- PM exposure to human primary human lung epithelial cells induces significant alterations in transcriptomic profile
 - Proinflammatory gene expression
 - Keratinization/cornification pathways
- PM exposure to human primary alveolar macrophages induces alterations in transcriptomic profile
 - Proinflammatory gene expression
 - NF κ B pathway
 - Fibrogenic gene expression (TGF- β , PDGF)
- Important implications for human pathology
- Identifies targetable pathways for therapeutic intervention

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