Role of Inhaled Pollutants in Risk of Pathogenic Infection

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Particulate matter pollution

- Hazardous PM
- Environmentally persistent free radicals (EPFRs)

Exposure to EPFRs increases severity of RTVI (infant/pediatric models)

- Epithelial injury
  - Immunosuppression
  - Failure to repair
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- Merck Investigators Studies Program
  - Targeting the viral and human host factors governing COVID19 disease to inform individual and population biomarkers of immunity and therapeutic development

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CENTRAL HYPOTHESIS

Adult respiratory diseases result, in part, from environmental insult(s) that occur during a critical phase of pulmonary immuno-maturation.

- Environment
- Viral
- Allergen

Diagram:
- RSV
- Allergen
- PM
- Influenza
- Adult Airways Disease
INFANTS HIGHLY VULNERABLE TO AIRBORNE EXPOSURES

Lungs & immune systems are still developing

High respiratory rate

Particle deposition is 35% higher compared to adolescents and adults (normalized to surface area)
Infant CD4+ T cells are poised for rapid Th2 effector function
- Hypomethylation (Hyper) at Th2 (Th1) cytokine regulatory region – CNS-1 (Rose et al. JI 2007)
- Secondary allergen challenge induces Th1 apoptosis (Li et al. Immunity 2004)

Secondary allergen challenge induces Th1 apoptosis (Li et al. Immunity 2004)

Infant CD8+ T responses are distinct from adults
- Neonatal CD8+ T cells are functionally immature (You et al. JI 2008)
- Require additional stimuli (McCarron et al. Hum Immunol 2010)
- Epitope dominance appears to be age-dependent (10 dpi; Ruckwardt et al. PLoS Pathogens 2011)
- Elevated Tc2 and Th2 cells elevated in infants w RSV severity (Siefker et al. 2020 AJRCCM)

Infant DCs
- Recruitment attenuated (Nelson et al. JI 1995; Upham et al. IAI 2006)
- Absolute number and subsets different (Cormier et al. JVI 2014)
- Diminished IL-12 synthesis (Lee et al. JEM 2007; Ripple et al. JI 2010)

Innate lymphoid cells
- IL33 responses are enhanced in infants (Saravia et al. PLoS Pathog 2015)
- Elevated ILC2 predicts severity (Vu et al. AJRCCM 2019)

RSV-specific IgA (Hijano et al. Scientific Reports 2018)
- Levels attenuated compared to adult
- Increased by IFNα supplementation to adult levels
THE BURDEN OF AIR POLLUTION

❖ Ambient air pollution is the 5th leading cause of death worldwide. Contributing to:
~ 4.2 million deaths worldwide
~ 400,000 deaths of children under five

*Health Effects Institute, 2019. State of Global Air*
NOT A LOCAL PROBLEM

Wildfire smoke map, 5:24 a.m. MDT September 4, 2017. The icons represent the locations of some of the large uncontained wildfires.
NOT JUST AN OUTDOOR CONCERN

EVERY YEAR, 1.96 MILLION PEOPLE DIE FROM ARIS AS A RESULT OF INDOOR AIR POLLUTION

Source: ARIAtlas.org, World Lung Foundation 2010

Grigg. 2011. Clinical & Experimental Allergy, 41: 1072-1075
COMBUSTION DERIVED PARTICULATE MATTER (PM)

Personal Sources (cigarettes)

Natural Sources (forest fires, volcanoes)
COMBUSTION-GENERATED PM CONTAINS RADICALS

Dellinger & Lomnicki
T.E. Sussan et al 2015
A. Valavanidis 2004

CS tar: 1e16 radicals/g
E-cig TPM: 2.6e15 radicals/g
PM$_{2.5}$: 1e16 - 1e17 radicals/g (BR); 1e17 - 1e19 radicals/g (Memphis)
Oyana TJ et al. ES&T. 2017
ENVIRONMENTALLY PERSISTENT FREE RADICALS (EPFRS)

\[ T_{1/2} = 21 \text{d} \]

CS tar: 1e16 radicals/g
E-cig TPM: 2.6e15 radicals/g
PM2.5: 1e16 - 1e17 radicals/g (BR); 1e17 – 1e19 radicals/g (Memphis)

Gehling et al. 2013
Sussan et al PLOS One 2015
Oyana TJ et al. ES&T. 2017
PERSISTENCE OF EPFRS IN BIOLOGICAL SOLUTIONS

Kelley et al., Chem Res Toxicol, 2013

Dugas

Cormier

Saravia et al., 2012
METAL OXIDE CONTENT AFFECTS LIFETIME OF EPFRS AND PCDD/F YIELDS

Metals affect EPFR lifetime and yield

Co-presence of metals affects both redox potential (not shown) and PCDD/F yields

Sprunger Lomnicki
Exposure to EPFRs increases severity of RTVI
POPULATION BASED PNEUMONIA STUDY

2358 total cases of radiographic pneumonia (all three sites)

167 (17%) not properly geocoded or not included in Memphis Metropolitan Area (MMA)

977 (41.4%) Pneumonia cases from Memphis

810 (83%)

387* (47.8%) with PLOS

114 (14.1%) admitted to ICU

CDC EPIC Cohort
Oyana et al., EBM 2021
COMMUNITY ACQUIRED PNEUMONIA (CAP) “HOTSPOTS”
SPATIAL PROFILES OF EPFRS IN MEMPHIS, TN

(a) Spatial Profiles of EPFRs in Memphis, Shelby County

Maximum spins/g values
- Less than or equal to 7.3638684E+17 (Low)
- 1.2286198511812E+18 (Medium)
- 3.09197541034129E+18 (High)
- More than or equal to 1.014573E+18 (Very High)

Sampling Locations of Leaf Data
- 509 sites
- 557 sites

(b) Spatial Profiles for Similar EPFRs

(c) EPA Tile Plot for PM2.5 Daily Air Quality Index
- Good (≤ 12.0 μg/m³) 282 days
- Moderate (12.0–35.4 μg/m³) 82 days
- Unhealthy for Sensitive Groups (35.5–55.4 μg/m³) 1 day
- Unhealthy (55.5–150.4 μg/m³) 0 day

Early November 2015 when leaf data was collected. It was rated moderate (12.0 to 35.4 μg/m³) 82 days.
Source: U.S. EPA AirData (https://www.epa.gov/air-data)
Created on December 6, 2016.
SPATIAL PROFILES OF EPFRS IN MEMPHIS, TN
PROXIMITY TO PM$_{2.5}$ SOURCES PREDICTS PNEUMONIA SEVERITY IN CHILDREN

❖ CAP has a non-homogenous geospatial distribution

❖ Higher than the mean PM$_{2.5}$ was associated with living in a high-risk area for CAP [adjusted odds ratio (aOR) 2.47, 95% confidence interval (CI) 1.31–4.66]
  ❖ Mean PM$_{2.5}$ (10.75 μg/m$^3$)

❖ Increased risk for CAP associated with
  ❖ Viral vs bacterial infection

❖ 1$^{st}$ to show possible PM$_{2.5}$ effects at exposure levels lower than the current EPA limit
  ❖ Recent reduction in WHO PM2.5 annual 5 μg/m$^3$ and 24h mean 15 μg/m$^3$

❖ Spatial overlap with high environmental EPFR concentration $^{Oyana et al. 2017, EST}$
EXPOSURE TO EPFRs INCREASES SEVERITY OF RTVI
EXPOSURE AND INFECTION PROTOCOL

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Flu Load

Viral Load

Lee, et al. PFT. 2014
EPFR EXPOSURE ENHANCES INFLUENZA MORTALITY

n = 16-35
EXPOSURE TO EPFRS SUPPRESSES PROTECTIVE IMMUNE RESPONSES
• Depletion of Tregs/IL10 in PM exposed mice increases protective T cell responses and reduces influenza morbidity & mortality
• IL10 alone recapitulates PM enhanced influenza morbidity
ROLE FOR IL22?

- Increase trans-epithelial resistance
- Epithelial cell proliferation
- Prevent secondary infection

Essential for damage repair after Flu infection

CONTINUOUS EPFR EXPOSURE RESULTS IN A FAILURE TO INDUCE IL22 EXPRESSION IN RESPONSE TO FLU

Kumar et al., PFT, 2021 In Press
RIL22 PROTECTS AGAINST PM INDUCED LUNG INJURY AND IMPROVES SURVIVAL IN FLU-INFECTED MICE

**Diagram A**
- Y-axis: Albumin (g/ml of BALF)
- X-axis: Time-point: 6 dpe (2 dpi)
- Comparison: Flu: + + +
- rIL22: - - +
- p = 0.093

**Diagram B**
- Y-axis: Percent survival
- X-axis: Days post-infection
- Lines:
  - Saline+Vehicle
  - Saline+rIL22
  - PM+Vehicle
  - PM+rIL22
- Annotations: a, b

rIL22 (5 ng/g body weight) 1dpe-2dpi
WHY DOES CONTINUOUS EPFR EXPOSURE RESULT IN A FAILURE TO INDUCE IL22 EXPRESSION IN RESPONSE TO FLU?
PM EXPOSURE INDUCES DYSBIOSIS OF LUNG (BALF) MICROBIOTA

(A) Shannon Index

(B) Relative abundance (%)

(C) Beta diversity (dissimilarity between sample pairs)

Alpha diversity (# of species in sample)
PM EXPOSURE DECREASES MICROBIAL-DERIVED INDOLE
Microbiome
  e.g., I3A
  PM
  AHR
  AhRE
  AhRE
  AhRE
  2kb
  IL22
  Flu
  • Epithelial repair
  • Protective adaptive immune responses
I3A PROTECTS AGAINST PM EXACERBATED LUNG INJURY AND FLU INDUCED MORTALITY

![Graph C](a graph showing albumin levels with time-point 6 dpe (2 dpi) and p=0.055)

![Graph D](a graph showing percent survival with days post-infection)

![Graph E](a graph showing IL22 expression normalized to Hprt with time-point 8 dpe (4 dpi))

**Flu:** + + +
**I3A:** - - +

**Flu:**
**Time-point:**
- 6 dpe (2 dpi)

**I3A:**
**Time-point:**
- 8 dpe (4 dpi)
**KEY RESULTS**

Exposure to EPFR containing PM

- Increased morbidity and mortality from influenza virus infection
  - Oxidative stress required
  - Supplementation with antioxidants reduced lung injury and improved survival

- Correlates with
  - Enhanced pneumonia risk in a pediatric population
  - Increased incidence respiratory tract infections in exposed communities

- Increased morbidity and mortality from influenza virus infection
  - Reduced IL22 following influenza infection
  - Supplementation with IL22 reduced lung injury

- Microbiome important in production of AHR ligands required for survival and maintenance of IL22
  - EPFR containing PM alter lung microbiome
  - I3A supplementation can protect against EPFR induced lung injury and Flu Induced Mortality

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Balakrishna S, et al. PFT. 2011;8:11
Oyana, et al. EST. 2017
Oyana, et al EBM. 2021
Kumar et al., PFT, 2021 In Press
IMPACT

Global Environmental Health

- Role of inhalation exposures in the exacerbation of respiratory tract infections
- Mechanisms by which PM exacerbates disease
- Potential therapeutic targets for attenuating PM-induced RTVI morbidity and mortality

Environmental Policy

- Enhance monitoring practices to include EPFRs
- Development of air quality standards for exposure to EPFR containing PM
QUESTIONS?

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