Towards Understanding the Immune Mechanisms of Air Pollution Associated Asthma

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Air Pollution & Asthma

- Ambient air pollution results in detrimental adverse health effects

**Deadly air pollution**

Air pollution killed around 7 million people worldwide in 2012 according to WHO’s latest report.

**Air pollution-linked deaths by region in millions**

- Indoor pollution: Western Pacific, South East Asia, Africa, Europe, Eastern Mediterranean, Americas
- Outdoor pollution: Western Pacific, South East Asia, Africa, Europe, Eastern Mediterranean, Americas

**Air pollution-linked deaths by disease in thousands**

- Ischaemic heart disease: 597
- Acute lower respiratory disease: 443
- Lung Cancer: 2,530
- Chronic obstructive pulmonary disease: 1,188
- Stroke: 2,297

Indoor pollution is mostly caused by cooking over coal, wood and biomass stoves.
Outdoor pollution is mostly caused by transport, power generation, industrial and agricultural emissions, and residential heating and cooking.

Source: World Health Organization

C. Inton, 26/03/2014

- AAP exacerbates asthma
- Immunopathology of AAP-induced asthma remains ill defined
Figure from Lambrecht and Hammad (2015)
Why focus on asthma and pollutions in the Central Valley in CA?

- 2005-2007: PM$_{2.5}$ exposure exceeded federal annual standard by >40%

- ALA 2014 pollution rankings (U.S. cities):
  - Short-term particulate pollution: 2$^{nd}$
  - Year-round particulate pollution: 5$^{th}$
  - Ozone pollution: 4$^{th}$

- Elevated rates of asthma (up to 21%) and allergies (up to 72%) in Fresno

_Tager, et al. FACES Report, CA Air Resources Board, 2010_  
_American Lung Association, State of the Air Report, 2014_  
_Nadeau, et al. JACI 2010._
Hypothesis

**Overall:** Treg are dysfunctional in subjects with underlying asthma and increased asthma exacerbations exposed to high levels of ambient air pollution exposure.

**Aim:** The research aims to help elucidate the role of T cells in air pollution-induced asthma exacerbations, a link which is theoretically understood, circumstantially clear, but not yet proven.

**Rationale:** By studying biological mechanisms, we improve our understanding of the pathophysiology of disease and develop methods to reduce the burden of asthma in children who are exposed to high levels of air pollution.
Ex vivo studies to determine whether a cause/effect relationship could exist

1. Purify human regulatory T cells by flow sorting to over 95% purity
2. Incubate with PAH (phenanthrene) vs diluent vs controls
3. Test if function, Foxp3, CpG methylation is changed
4. If so, how? Via DNMT? Via AhR? Other?
5. Use inhibitors to test mechanisms
PAHs modulate T cells → Th17 or Treg?

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

6-formylindolo-[3,1-b]carbazole (FICZ)

Phenanthrene (Phe)

T lymphocyte

AhR

Quintana et al., 2008, 2010
Veldhoen et al., 2008, 2009
Kimura et al., 2008
Results

Phenanthrene exposure increased CpG methylation in Tregs

Results

Phenanthrene exposure impaired Treg function

Results

Phe induce IL-4, pSTAT6, GATA3, IL-13

Results

AhR, DNMT1, and DNMT3b mediated phenanthrene’s effects on Treg

In vivo: Four cohorts chosen:

Methods

• 1) Children’s Environmental Study or Asthma HPE
  • 9 years of exposure data
  • Children 8-12 yrs with asthma
  • 315 families
  • Serial PFTs and clinical outcome score
  • Viral illnesses, allergens, stress

• 2) Fresno control or Healthy HPE: Age matched and sex matched children with no asthma and no allergies

• 3) Stanford Asthma or Asthma LPE: Age matched and sex matched children with asthma (same clinical outcomes and PFTs used)

• 4) Stanford control or Healthy LPE: Age matched and sex matched children with no asthma and no allergies

Note: Stanford group must live 6 yrs or more at residence in Palo Alto, CA and must live 30 meters outside range of major highway

Lurmann, Nadeau, Mann, Noth, Tager, Hammond, Balmes, UCB Public Health School
Methods

Enrolled 552 children to date

- Blood samples (n=402)
  - Plasma
  - PBMCS
- Saliva samples (n=113)
  - Microbiota
- Urine samples (n=246)
  - 4 Parent PAH classes with CDC

Individual estimate air pollution exposures

Spirometry
Questionnaires
Methods

• Multi-pollutant exposure measurement
  – use a spatial-temporal regression model
  – incorporates both temporal and spatial covariates into the exposure estimates for each individual
  – Simultaneously measure 7 AAPs, O$_3$, EC, NO, NO$_2$, PM$_{10}$, PM$_{2.5}$, and PAH
  – Over 12mon, 6mon, 3mon, 1mon

A spatial-temporal regression model to predict daily outdoor residential PAH concentrations in an epidemiologic study in Fresno, CA

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Atmos Environ 2011
**New technology-assisted systematic study:**

**Multiplex Immunophenotyping reveals Effects of Discrete Environmental Exposures on the Innate and Adaptive immune system**

1. **Apply mass cytometry by time-of-flight (CyTOF) method to profile multiple immunophenotypes in parallel and in single cells**

2. **Measure seven different ambient air pollutants at four different time points through a year**

3. **Define the association between exposure level and immunophenotypes**
Time of Flight Mass Cytometry (Cytof): Surpassing Flow Cytometry

- No spectral overlap or compensation issues as in flow cytometry.
- Able to detect up 30 to 40 parameters simultaneously.
- Smaller blood volume required.
Methods

Blood

Lymphoid cells

Myeloid cells

T reg
CD4
CD25hi
CD127lo

CD4+
(effector cells)
CD4
CD25lo
Or
CD25-

Th2
CD4
CD294

Th17
CD4
IL-17

B cell
CD4
CD19
CD20
CD27

NK/T cell
CD56
CD16

Dendritic Cells
CD123
CD303
CD11c
CD1c

Monocytes
CD14
CD16

Eosinophils
CD66bhi
CD16lo

Basophils
2D7
FcεRI
CD203hi

Molecules expressed on cell surface with CCR4, CCR8, CD45RO, CD45RA, etc.
Intracellular markers to be stained: Ki67, CD69, IL-9, pSTAT5, CCL1, CCL22, CCL17, IL-4, IL-13, IL-10, TGF-β, IL-17, IFN-γ, etc.

STANFORD UNIVERSITY  Bendell. et al. Science 2011
Results

• Phenotypic diversity of the four groups in the study
Results
--in healthy

- Visualize differential immune cell subsets between HPE and LPE in healthy

a: “alternative” monocytes, CD14dim/-CD16+; b: “classical” monocytes, CD14+CD16-; c: CD14-CD16+CD33- cells; d: CD4+ T cells; e: CD8+ T cells; f: B cells, CD20+. 
Results

--in asthma

- Visualize differential immune cell subsets between HPE and LPE in asthma

a: “classical” monocytes, CD33+CD14++CD16-; b: “alternative” monocytes, CD33+CD14dim/-CD16+; c: CD33-CD14-CD16+; d: CD4+ T cells; e: CD8+ T cells; f: B cells, CD20+. 
Differentially Expressed Immune Cell Subsets by Air Pollution and by Disease

- Immunophenotypes significantly ($p < 0.05$) linked with LPE or HPE in healthy or disease (asthma) are positioned in a quadrant plot.

- The horizontal axis indicates the magnitude of regression coefficient used to separate HPE and LPE subjects.

- The vertical axis indicates the asthmatic condition in which the regression coefficient is found significant. The position on the vertical axis corresponds to the magnitude on the horizontal axis.
Reduced Anti-tetanus Immunity in Healthy

- The IgG tetanus toxoid-specific antibodies were measured by ELISA in the healthy subjects with low polluted environment (LPE) or high polluted environment (HPE)

- A lower response was observed in healthy subjects with HPE compared to healthy subjects with LPE ($p=0.017$)
Conclusion

• Treg dysfunction and Foxp3 epigenetic changes are associated with increasing levels of polycyclic aromatic hydrocarbon in asthmatic patients, during chronic exposure.

• Specific T cells were purified in order to find these epigenetic changes.

• In vitro studies demonstrate a possible link with polycyclic aromatic hydrocarbon (i.e. phenanthrene) exposure and aryl hydrocarbon receptor-mediated epigenetic changes in Treg.

• Multiplex immunophenotyping is a potential tool to identify specific immune cell types and their response to environmental exposures.
With Appreciation to EPA/NIEHS 2015, Patients and Families, Clinical and Laboratory Team and Collaborators