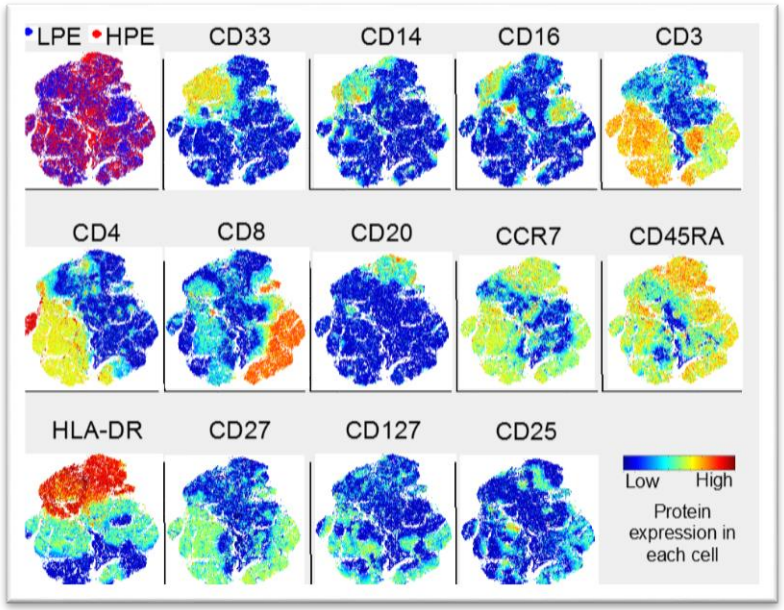
Towards Understanding the Immune Mechanisms of Air Pollution Associated Asthma



Kari Nadeau, MD, PhD

Director, Sean N. Parker Center for Allergy & Asthma Research at Stanford University

Naddisy Family Foundation Professor of Medicine

NIEHS/EPA Webinar

October 14, 2015

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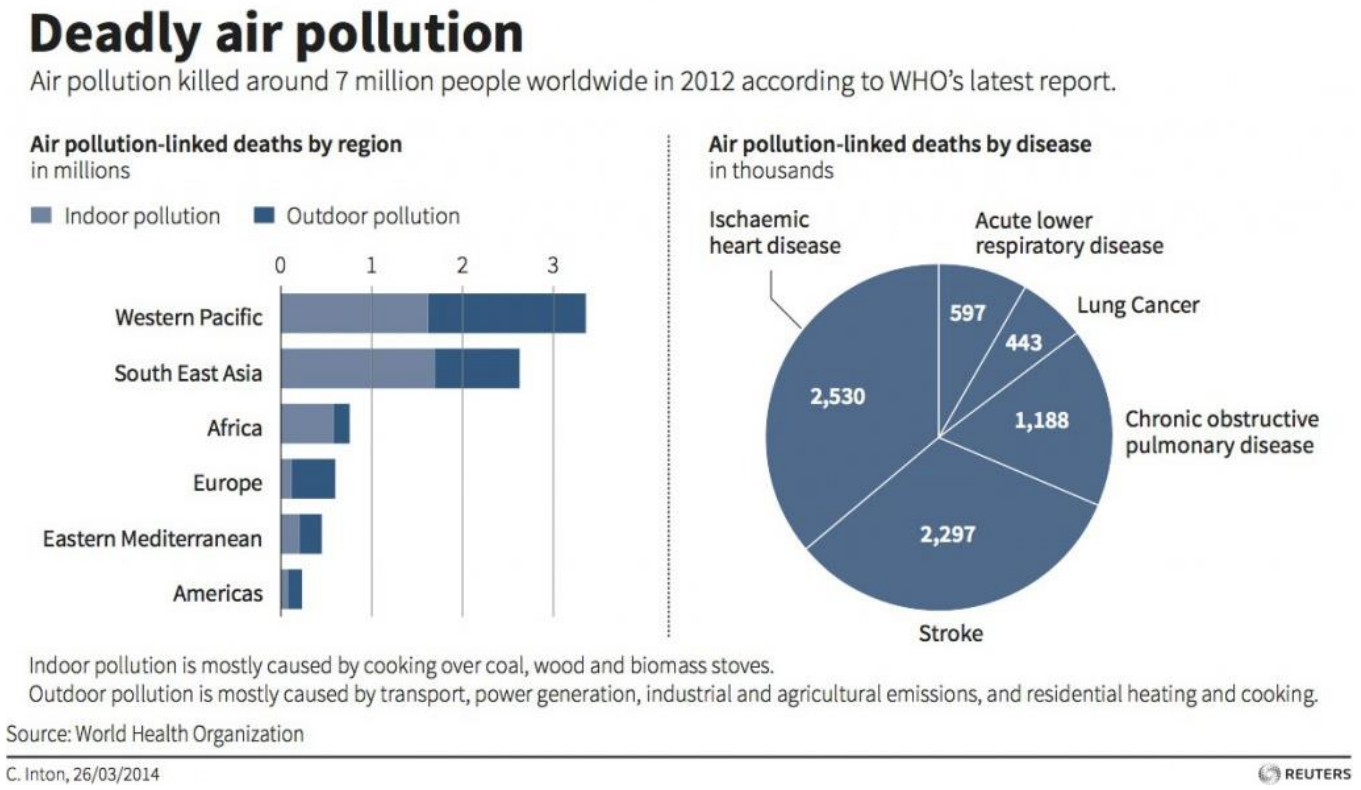
Air Pollution & Asthma

- Ambient air pollution results in detrimental adverse health effects



- AAP exacerbates asthma
- Immunopathology of AAP-induced asthma remains ill defined

Immune System & Asthma

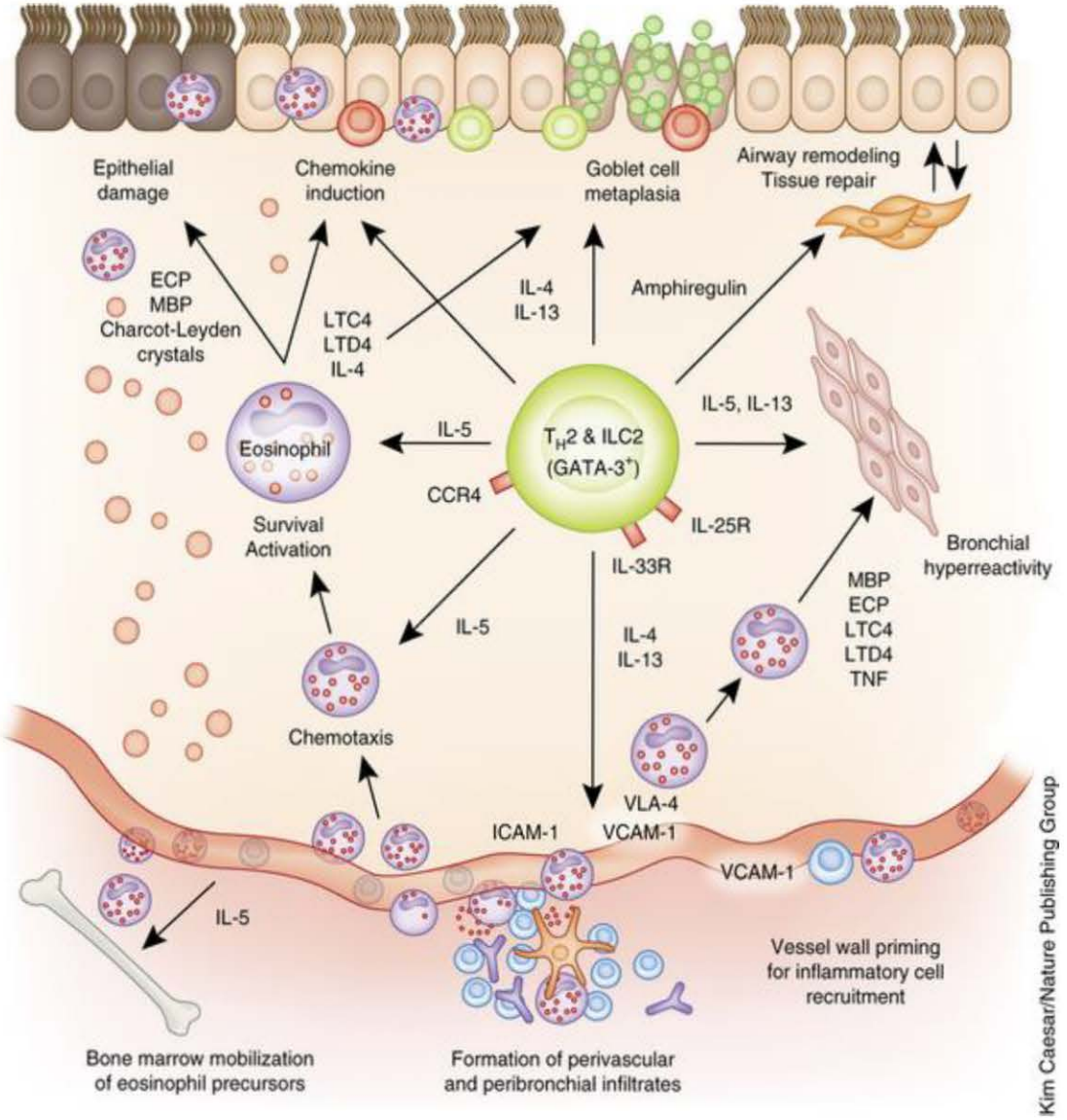


Figure from Lambrecht and Hammad (2015)

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Why focus on asthma and pollutions in the Central Valley in CA?

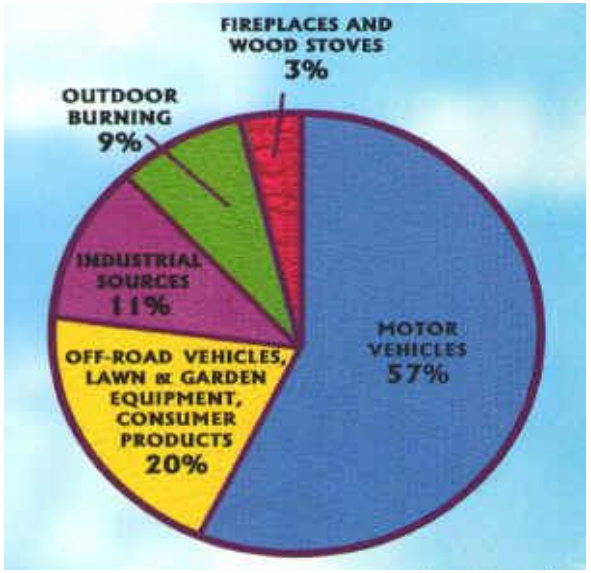
- 2005-2007: $PM_{2.5}$ exposure exceeded federal annual standard by >40%

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

- 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.

Sources of Air Pollution in Fresno



American Lung Association, California Air Resources Board, California Health Interview Survey

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.

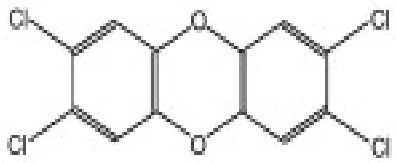
Rationale: By studying biological mechanisms, we improve our understanding of the pathophysiology of disease and develop methods to reduce the burden of asthma in children who are exposed to high levels of air pollution.

Background/Methods

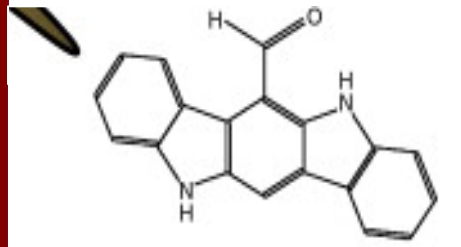
***Ex vivo* studies to determine whether a cause/effect relationship could exist**

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*

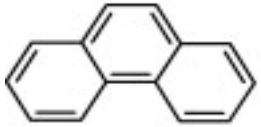
PAHs modulate T cells→Th17 or Treg?



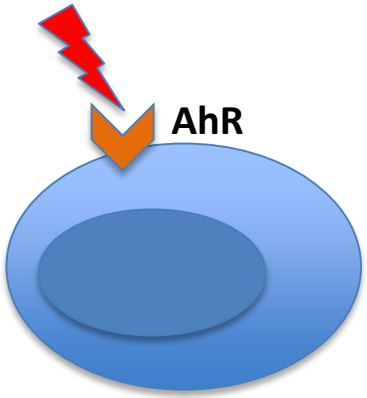
2,3,7,8-tetrachlorodibenzo-p-dioxin
(TCDD)



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



Phenanthrene
(Phe)



T lymphocyte

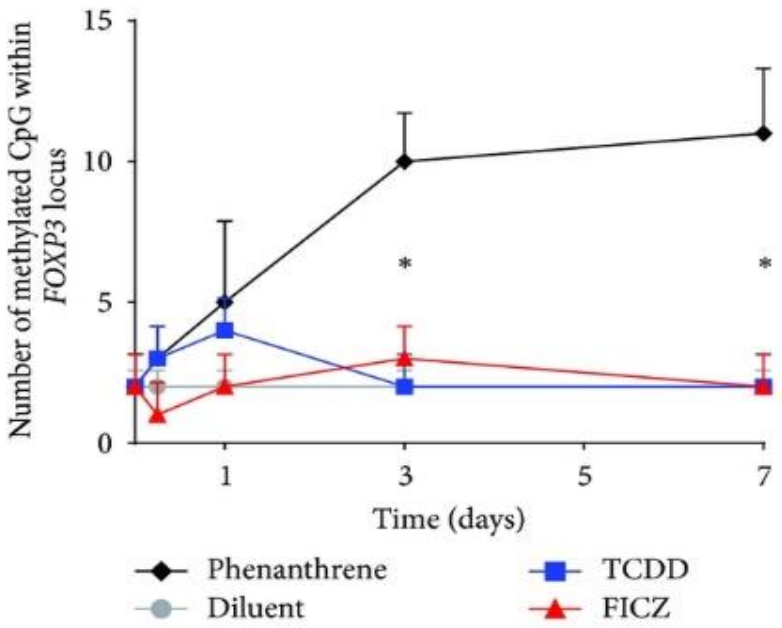
Treg — Quintana et al., 2008, 2010

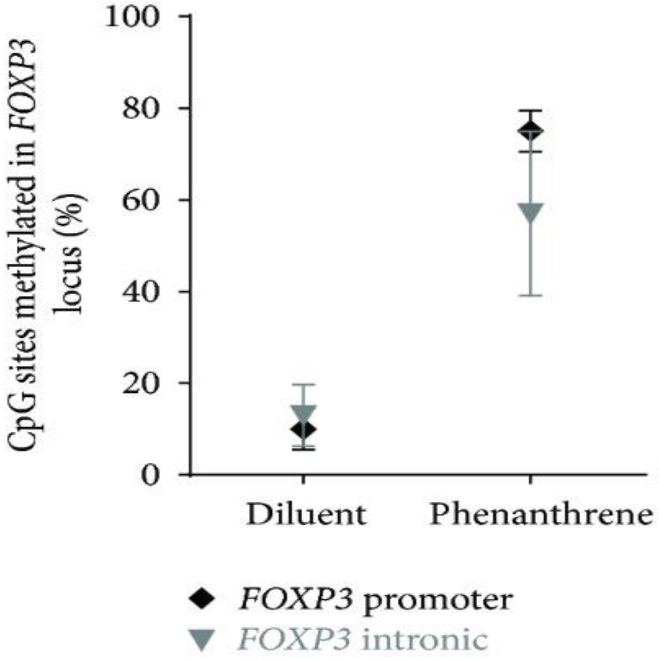
Th17 — Veldhoen et al., 2008, 2009

Th2 — Kimura et al., 2008

Results

Phenanthrene exposure increased CpG methylation in Tregs

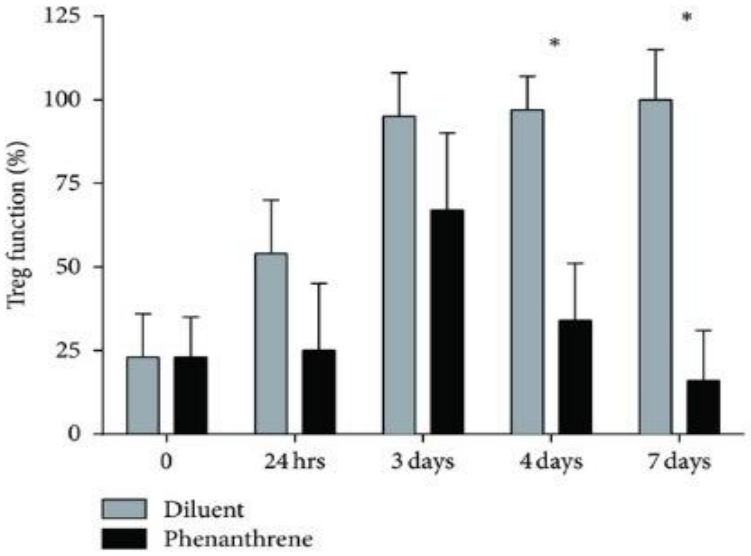


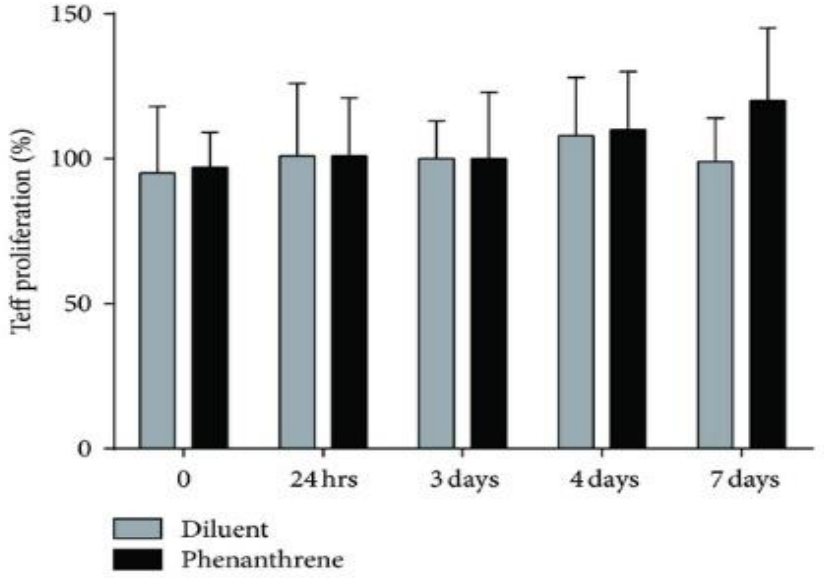


Liu J, Zhang L, Winterroth LC, Garcia M, et al. Journal of Toxicology. 2013.

Results

Phenanthrene exposure impaired Treg function

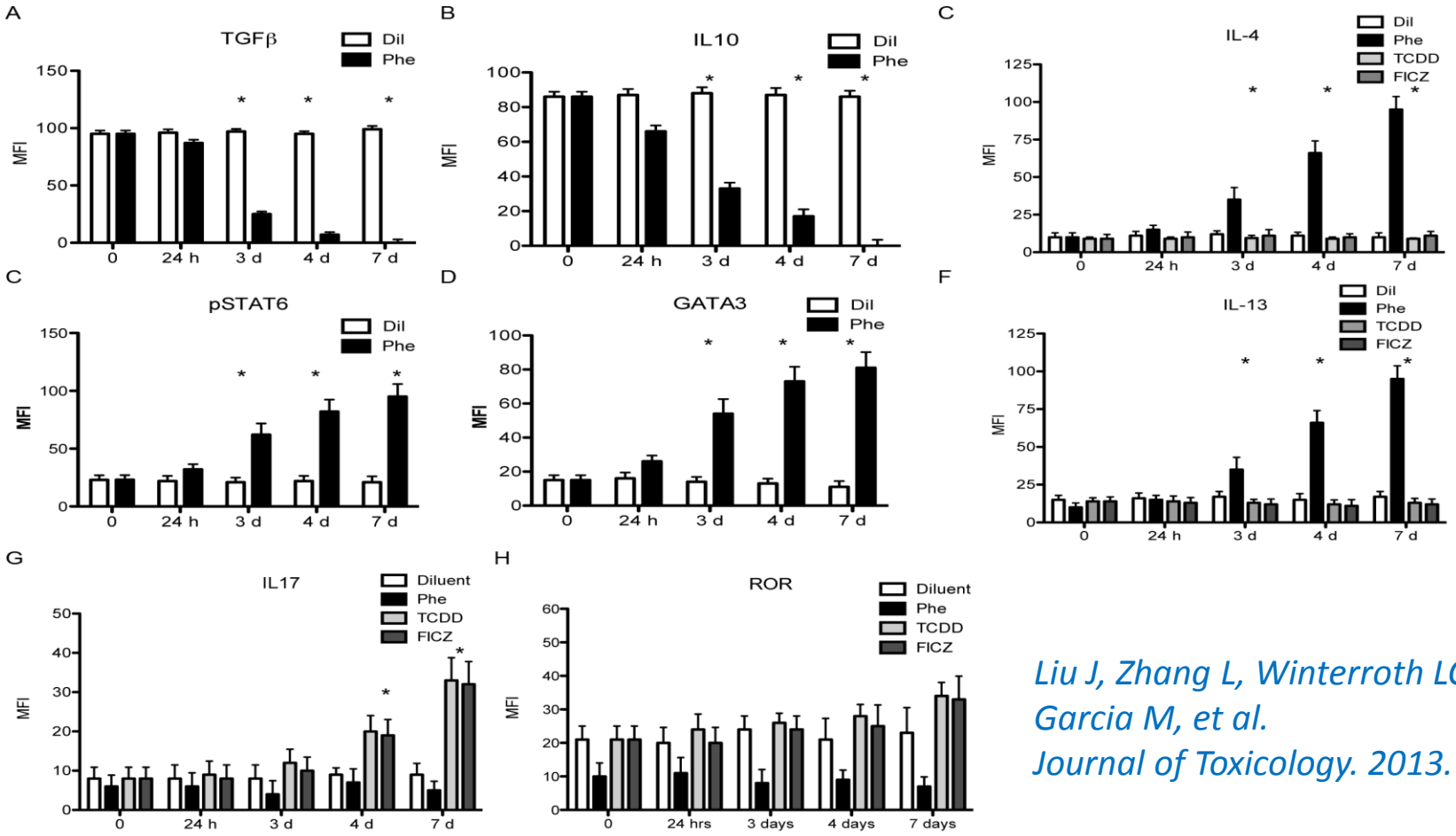




Liu J, Zhang L, Winterroth LC, Garcia M, et al. Journal of Toxicology. 2013.

Results

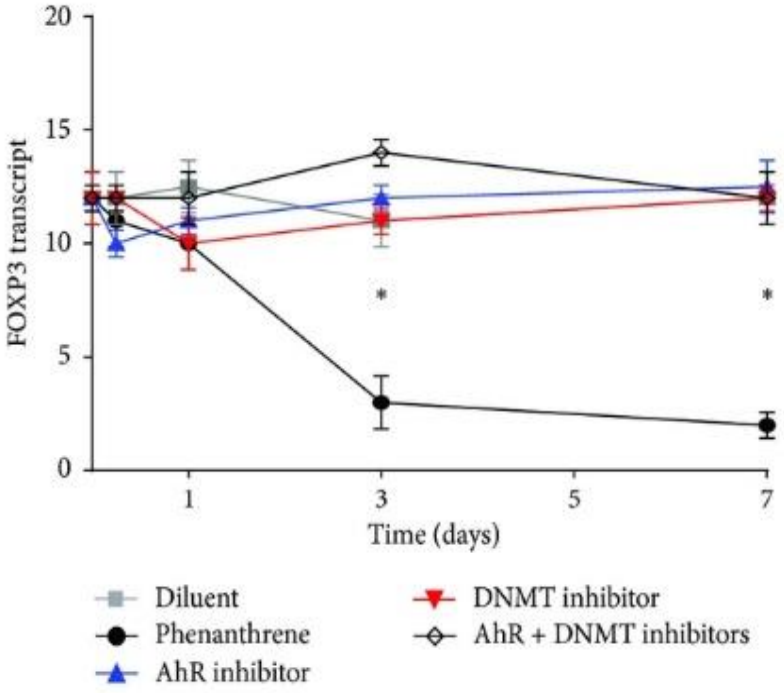
Phe induce IL-4, pSTAT6, GATA3, IL-13

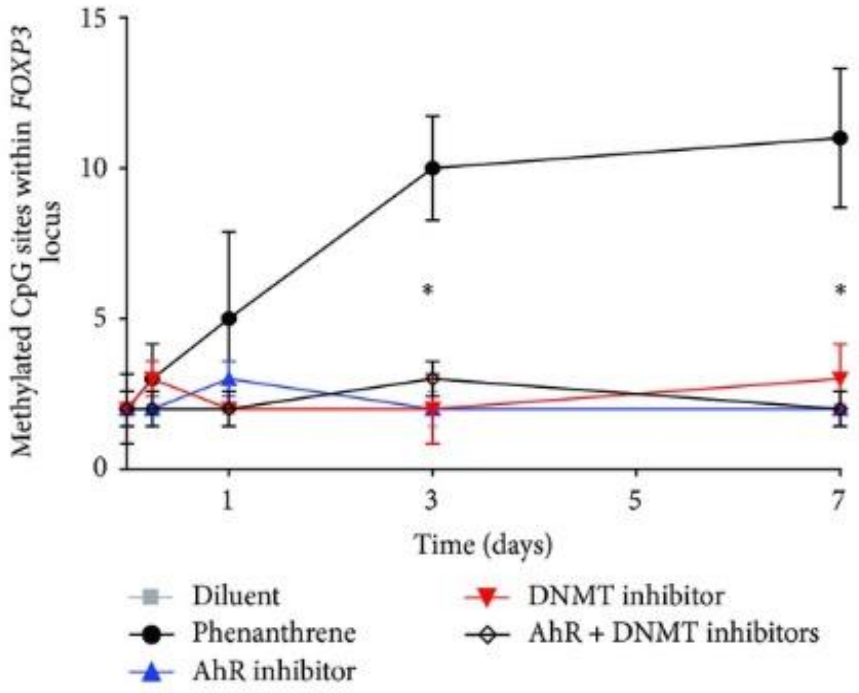


Liu J, Zhang L, Winterroth LC, Garcia M, et al.
Journal of Toxicology. 2013.

Results

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





Liu J, Zhang L, Winterroth LC, Garcia M, et al. Journal of Toxicology. 2013.

In vivo: Four cohorts chosen:

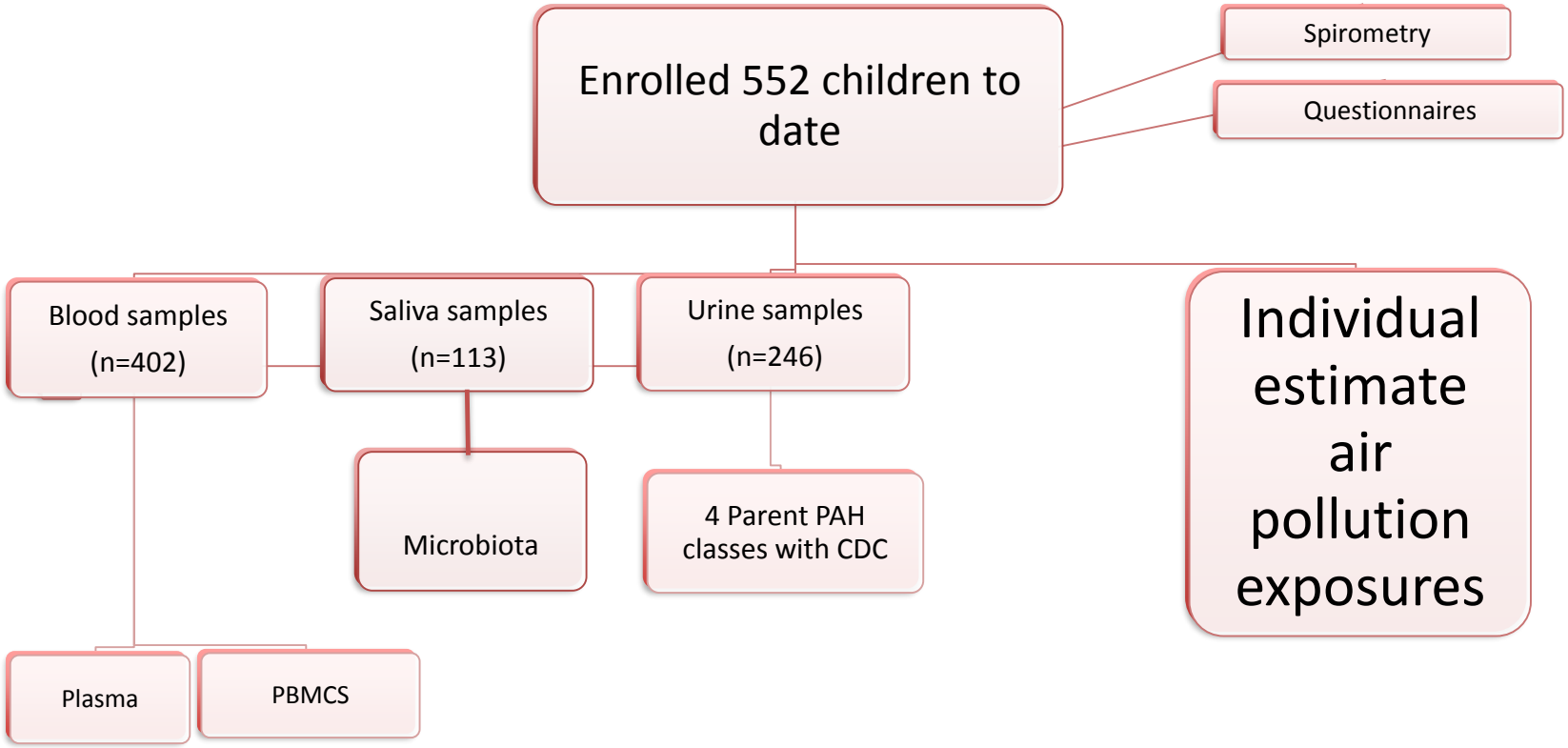
Methods

- 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



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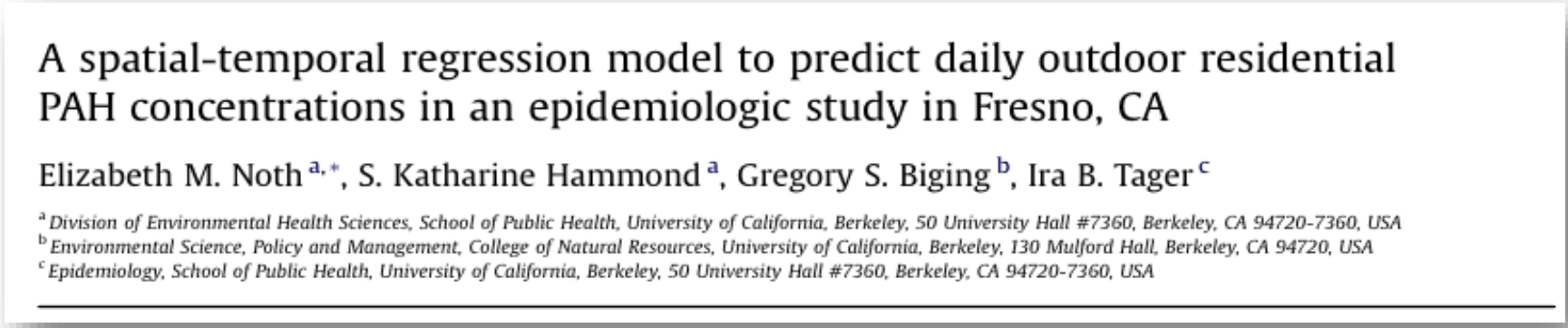
Methods

- 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

Elizabeth M. Noth [a,*], S. Katharine Hammond [a], Gregory S. Biging [b], Ira B. Tager [c]

[a] Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, 50 University Hall #7360, Berkeley, CA 94720-7360, USA
[b] Environmental Science, Policy and Management, College of Natural Resources, University of California, Berkeley, 130 Mulford Hall, Berkeley, CA 94720, USA
[c] Epidemiology, School of Public Health, University of California, Berkeley, 50 University Hall #7360, Berkeley, CA 94720-7360, USA

Atmos Environ 2011

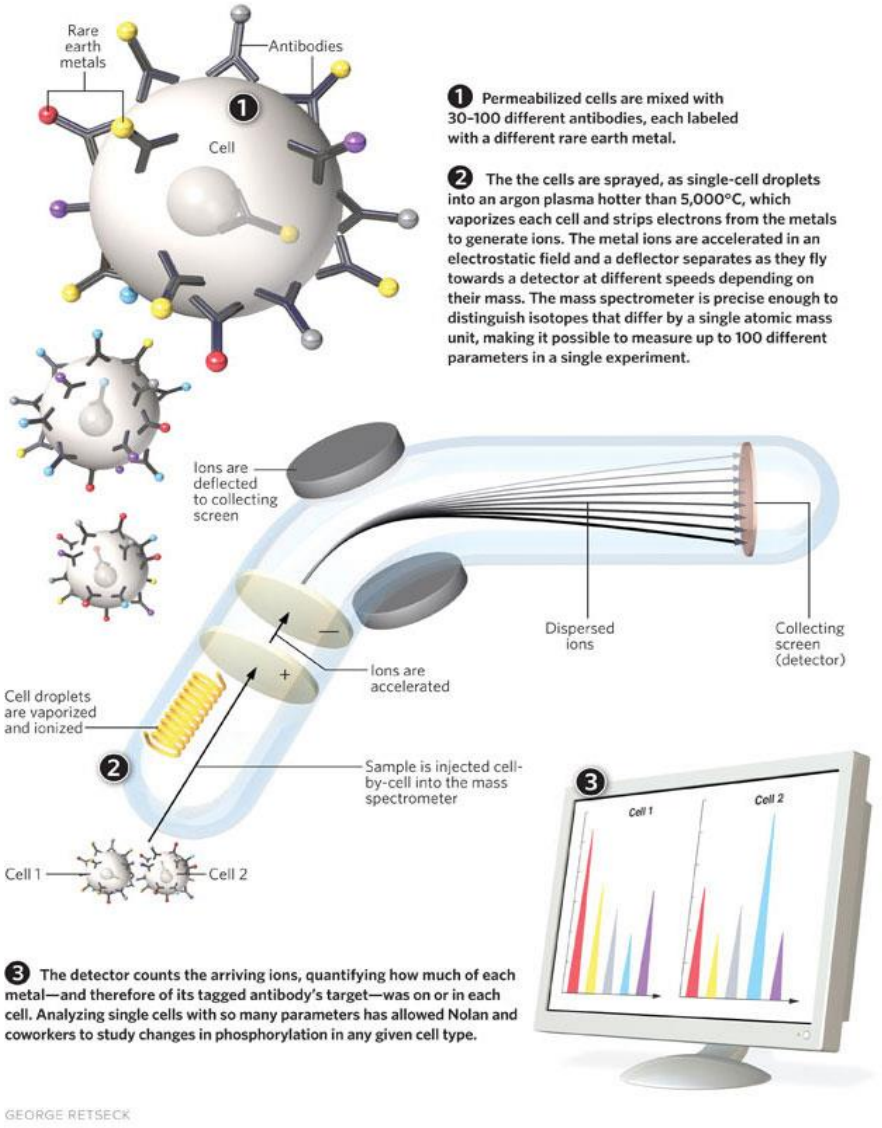
New technology-assisted systematic study:

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

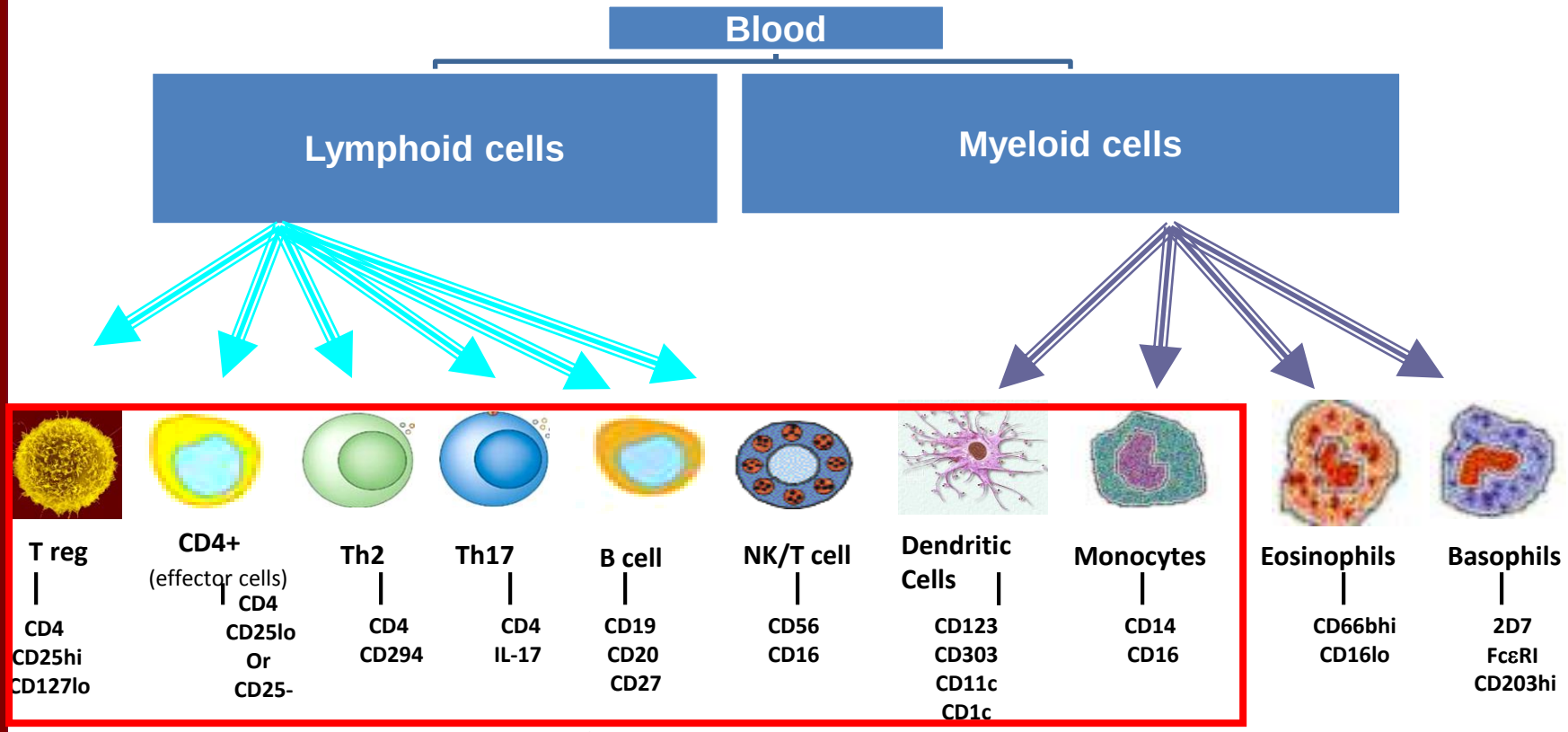
1. *Apply mass cytometry by time-of-flight (CyTOF) method to profile multiple immunophenotypes in parallel and in single cells*
2. *Measure seven different ambient air pollutants at four different time points through a year*
3. *Define the association between exposure level and immunophenotypes*

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



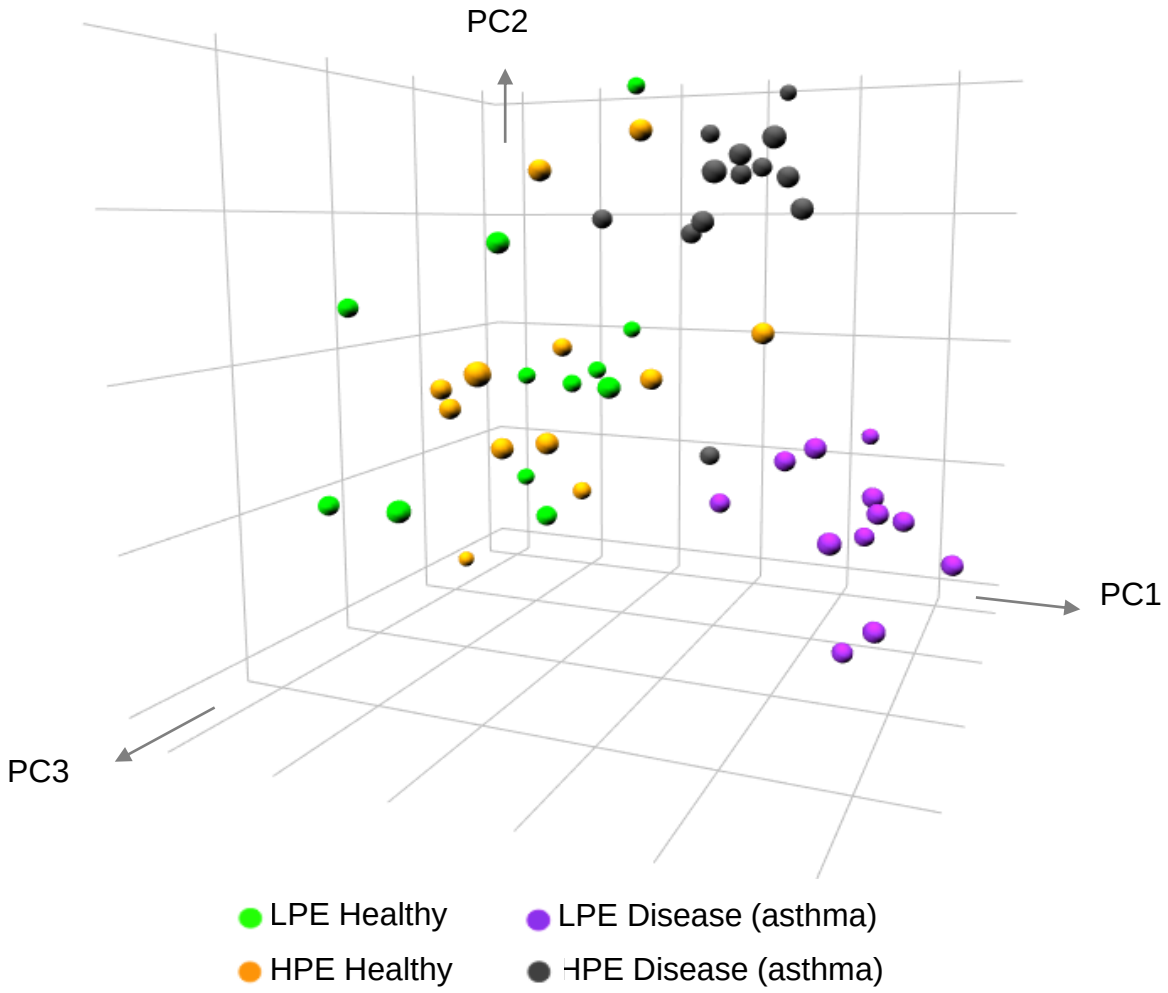
Molecules expressed on cell surface with CCR4, CCR8, CD45RO, CD45RA, etc.

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

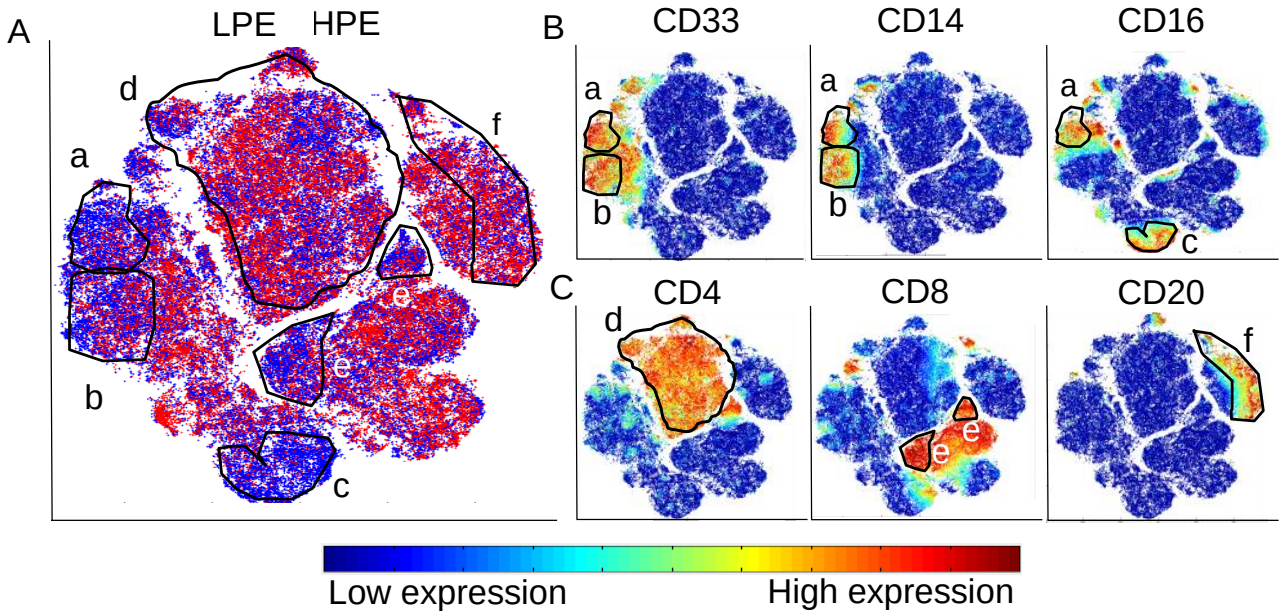
- Phenotypic diversity of the four groups in the study



Results

--in healthy

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

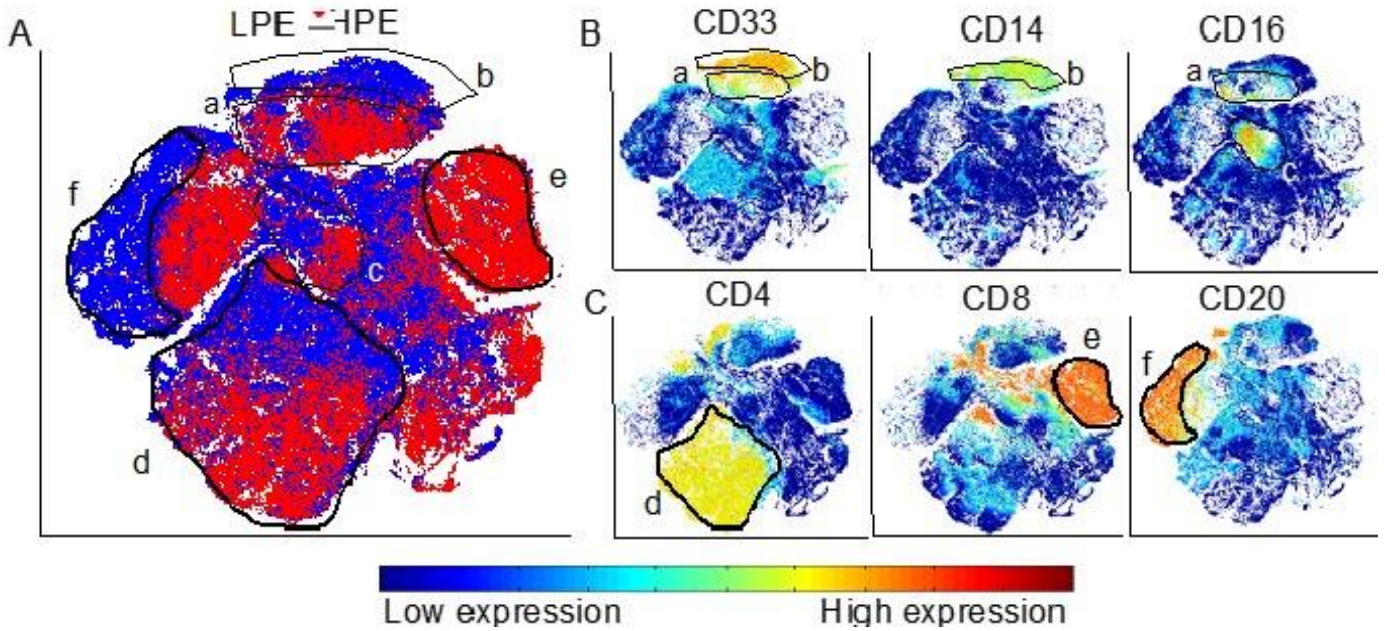


a: “alternative” monocytes, CD14dim/-CD16+; b: “classical” monocytes, CD14+CD16-; c: CD14-CD16+CD33- cells; d: CD4+ T cells; e: CD8+ T cells; f: B cells, CD20+.

Results

--in asthma

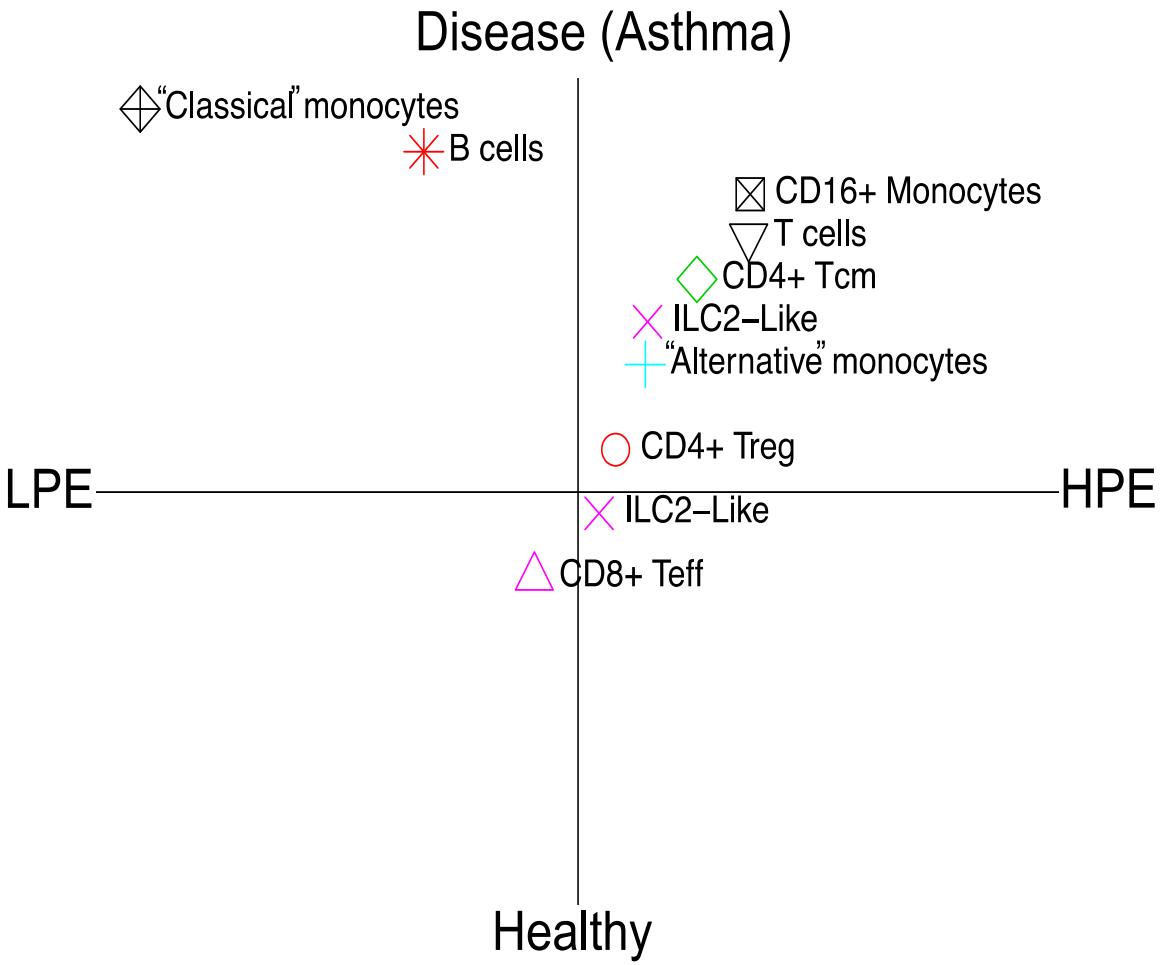
- Visualize differential immune cell subsets between HPE and LPE in asthma



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+.

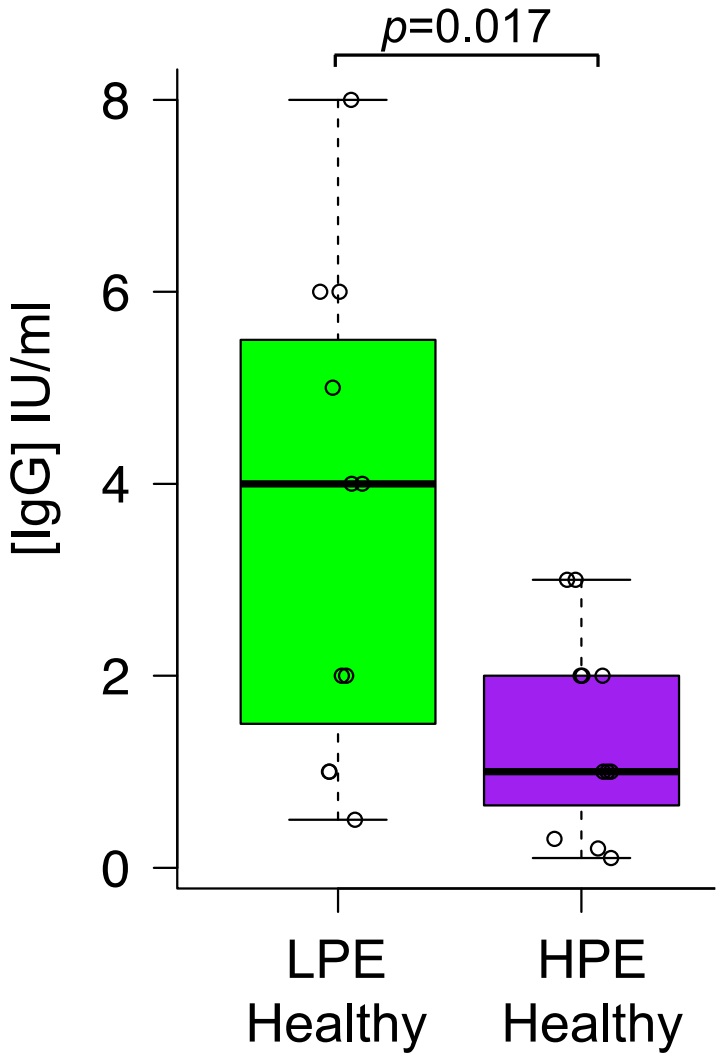
Differentially Expressed Immune Cell Subsets by Air Pollution and by Disease

- 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 horizonal 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

NIEHS
National Institute of Environmental Health Sciences

National Institute of Allergy and Infectious Diseases

NIH National Heart, Lung, and Blood Institute

EPA
United States Environmental Protection Agency

AAAAI
American Academy of Allergy Asthma & Immunology

Immune Tolerance Network

FARE
Food Allergy Research & Education

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