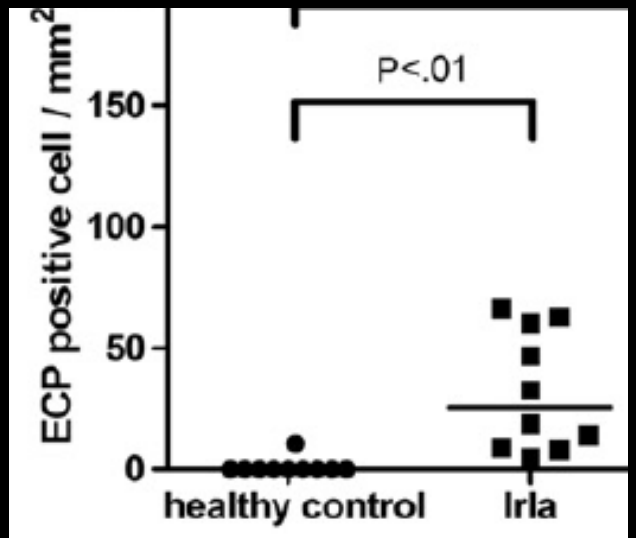
Long-term pathologic consequences of acute irritant-induced asthma

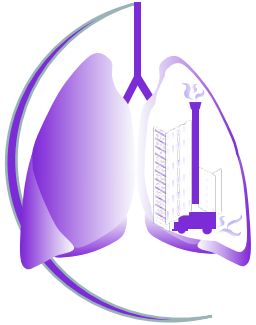
Naoya Takeda, MD, PhD,[a] Karim Maghni, DSc, PhD,[b] Sylvie Daigle, RN,[b] Jocelyne L'Archevêque, RT,[b] Lucero Castellanos, RT,[b] Wisam Al-Ramli, BSc,[a] Jean-Luc Malo, MD,[b] and Qutayba Hamid, MD, PhD[a] *Montreal, Quebec, Canada*

(J Allergy Clin Immunol 2009;124:975-81.)

Bronchoalveolar lavage fluid analysis showed increased eosinophil in 30% of subjects with IrIa

BAL	IrIa (n = 10)	Healthy subjects (n = 10)	Subjects with asthma (n = 10)
ECP (ng/mL)	1.93 (1.43-6.28)	* .001	0.13 (0.05-3.76)





UBC Chan-Yeung Centre for Occupational and Environmental Respiratory Disease

Understanding exposure effects – linking research to public health

APEL Air Pollution Exposure Laboratory

UBC

6th Jack Pepys Workshop
TORONTO
May 2016

How does exposure to gas, dust and fumes enhance (allergic) sensitization and asthma?

Chris Carlsten

Associate Professor of Medicine
Astra-Zeneca/Canada Research Chair in Occupational & Environmental Lung Disease
University of British Columbia

No conflicts to disclose

Why do we care about mechanisms?

- Target prevention
- Target treatment
- Inform guidelines or regulations
- Support compensation claims
- Increase basic understanding of biology
- Potential application to other settings

Sensitization *versus* asthma

Sensitization

- Specific; adaptive immunity
- Associated with a range of 'atopic' disease

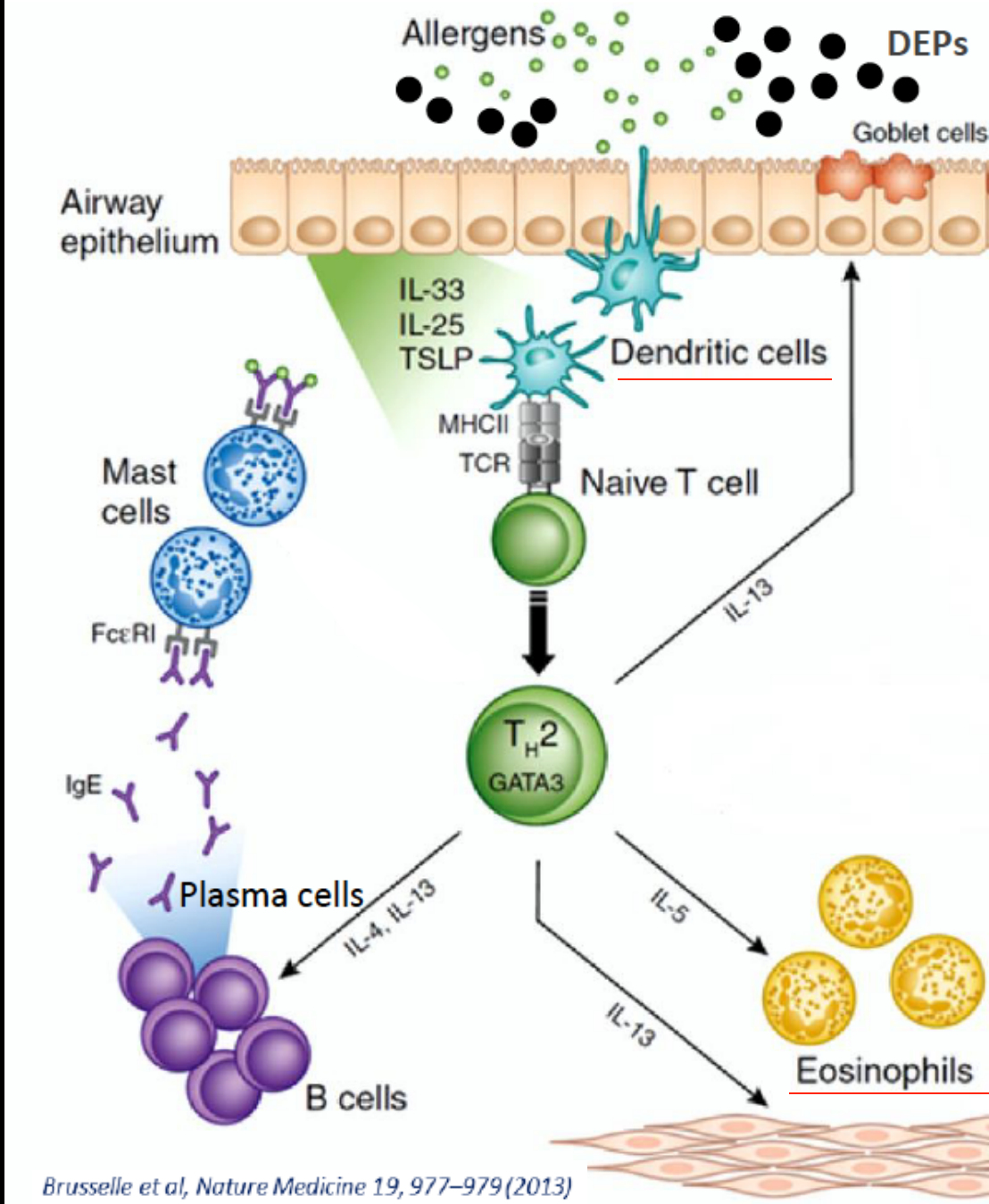
Asthma

- Clinical phenotype supported by lung function
- May be via sensitization or otherwise

"Enhance" – exacerbate or ⬆ incidence

Possible mechanisms 'where?'

- Cells within airway lumen
- Epithelium (+/- damage)
- Subepithelium
- Circulation

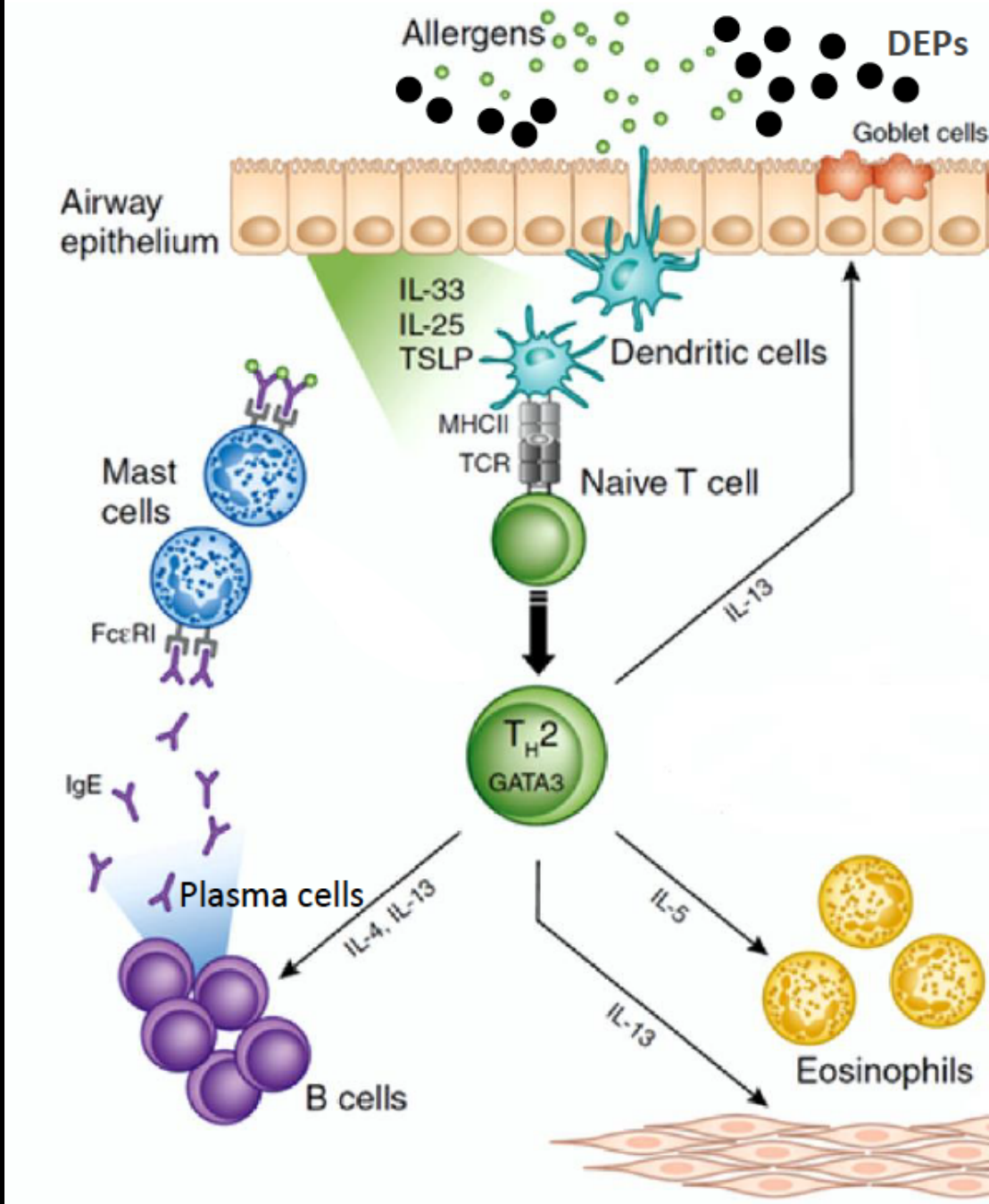


Possible mechanisms
'how?'

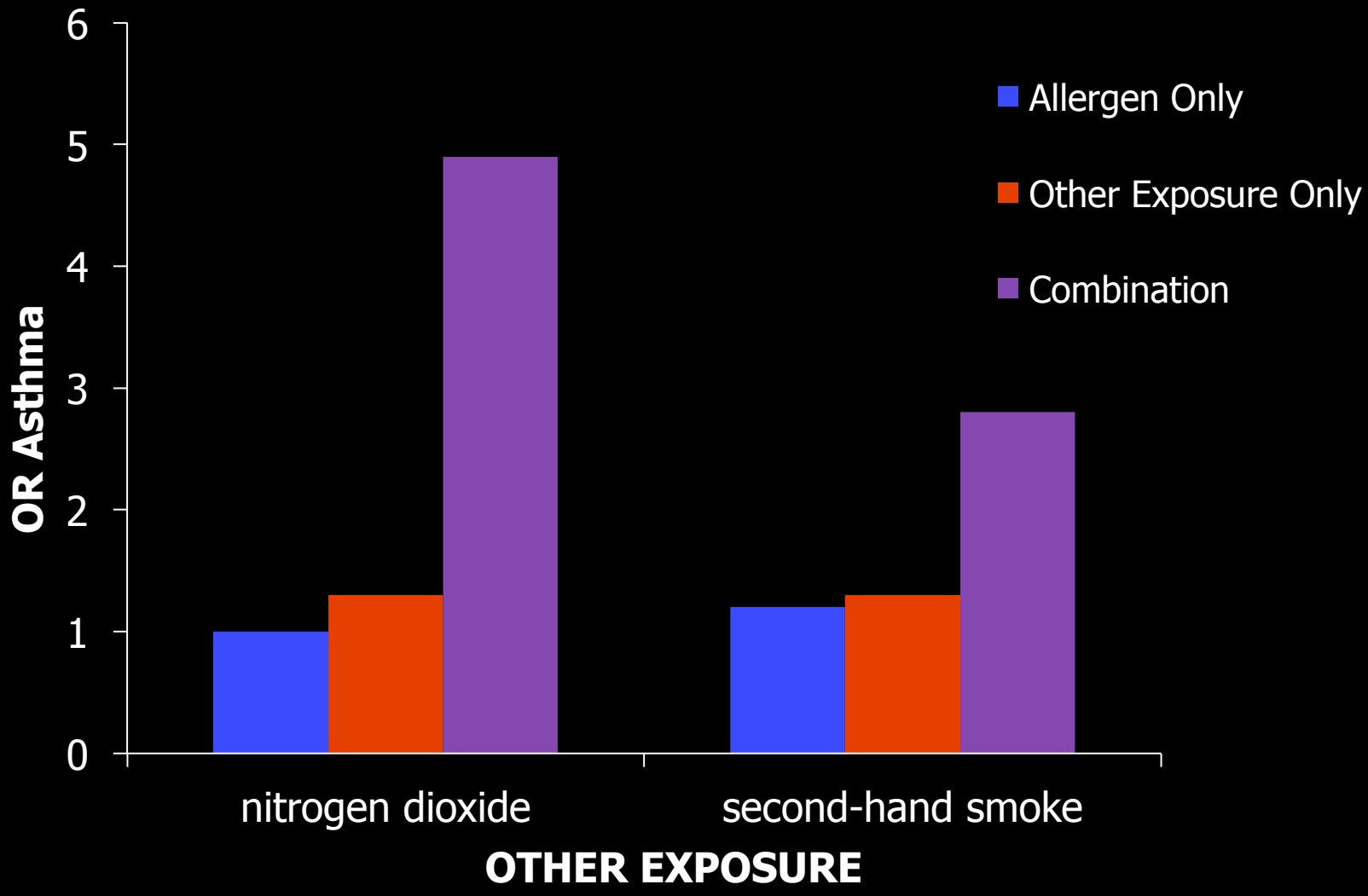
Classic cells and mediators

Novel mediators
- Transcriptome
- Proteome
- Epigenome

Modifying factors (gene variants)



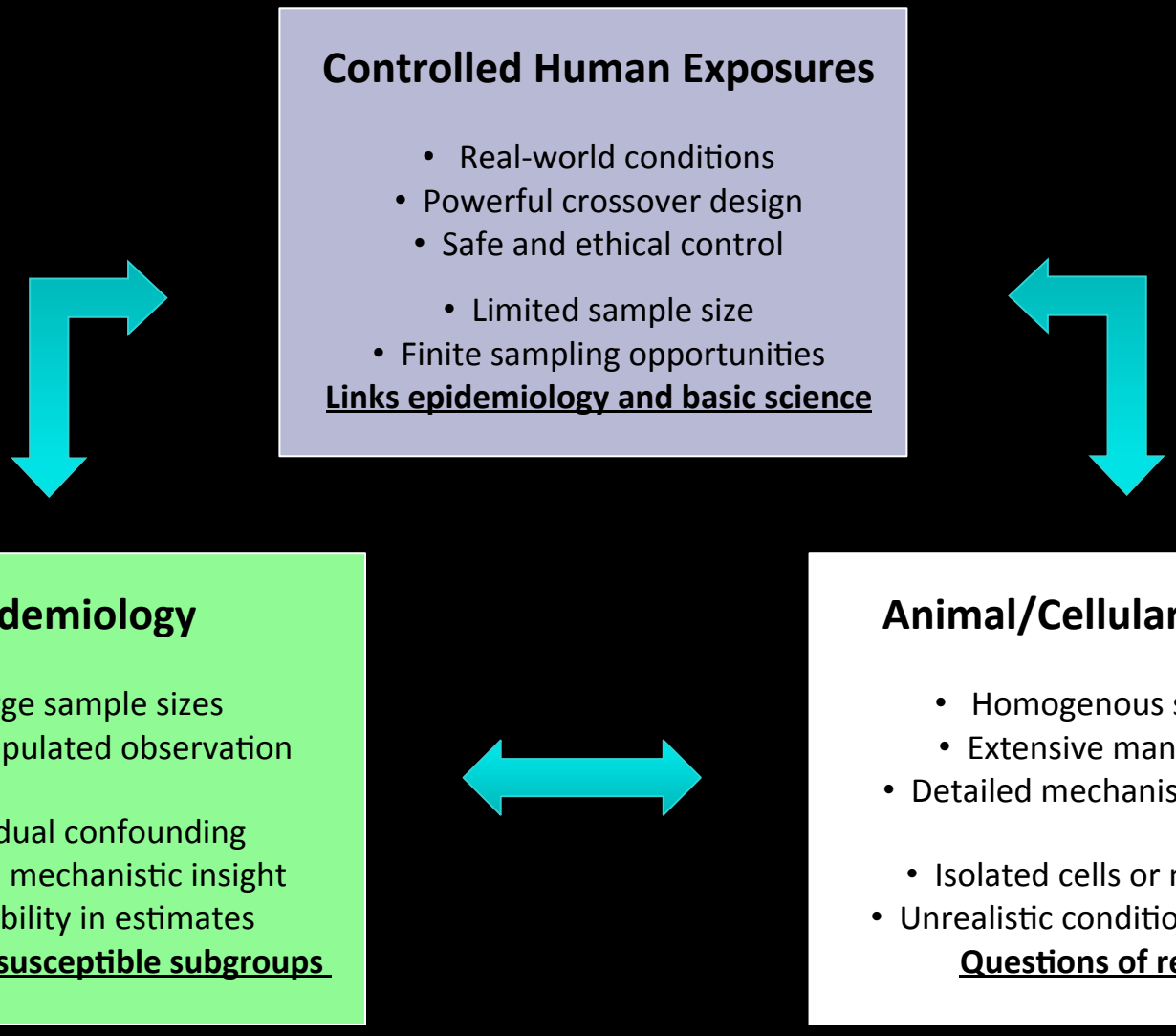
Synergistic early life co-exposures
Incident asthma (age 8)



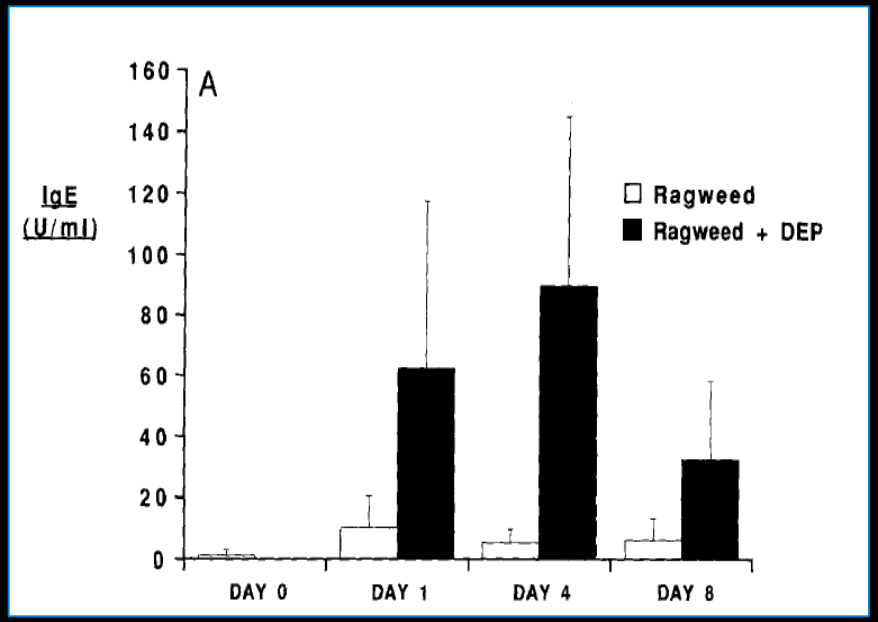
Carlsten *ERJ* 2010 PMID: 20530047

3 pillars of plausibility

Carlsten and Georas, *AJRCCM* 2014 PMID: 24787066



PM plus allergen: ↑ specific IgE in the human nose



Diaz-Sanchez -- J Immunol 1997

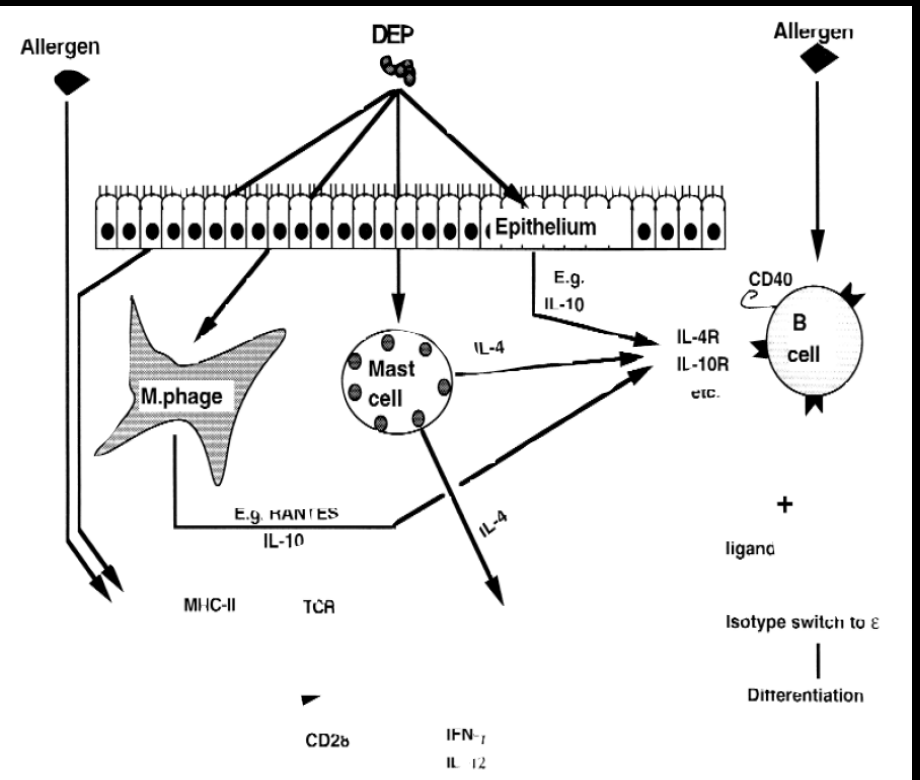
	GSTP1	
	I/I (n=13)	I/V (n=6)
Ag alone	7·8 (3·2–24·3)	8·4 (2·6–18·8)
DEP+Ag	123·5 (14·5–534·8)	31·5 (8·8–79·4)

Gilliland -- Lancet 2004

Repeated dosing of 300 ug DEP → IgE to NEOantigen (JACI PMID: 10588999)

Possible mechanisms

Nel, Diaz-Sanchez *JACI* 1998

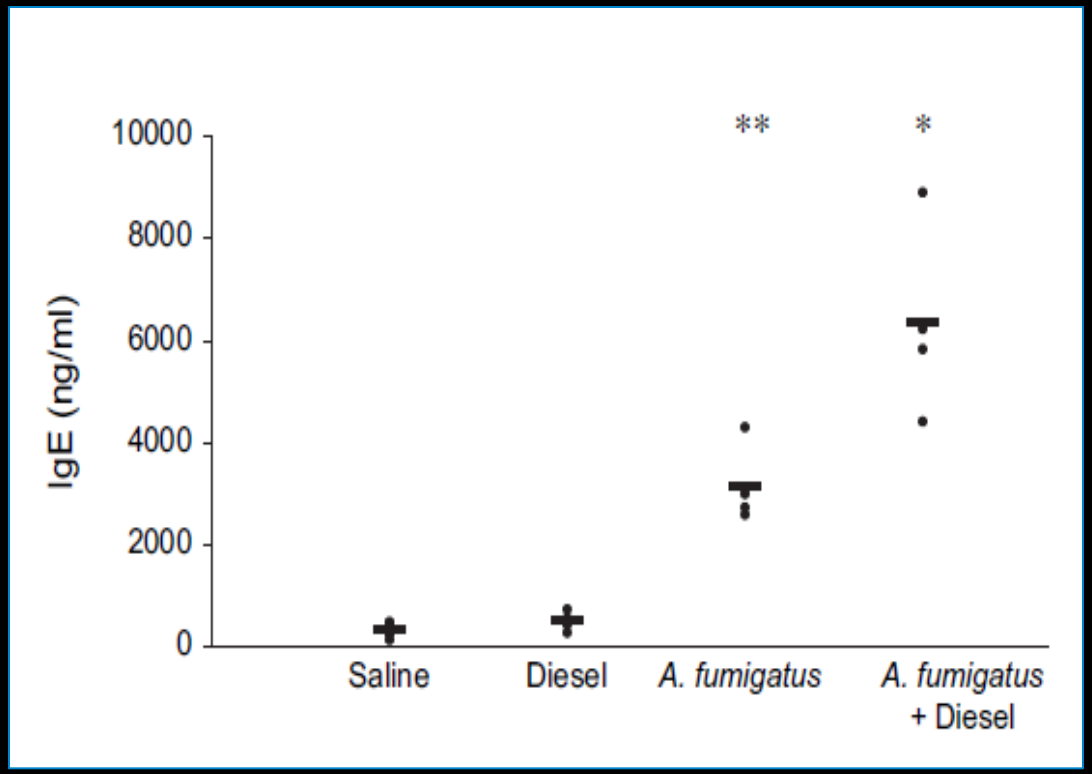


Effects of aerosolized DEPs on nasal allergic response

- Increased local production of IgE and IgE-secreting cells
- Qualitatively different IgE isoforms produced through alternative splicing of ε mRNA
- Augmented production of allergen-induced antigen-specific IgE
- Non-specific stimulation of a broad cytokine profile in the absence of allergen
- Induction of a Th2-like cytokine profile when intranasally administered together with allergen in the nose
- Interact with allergen to drive isotype switching of B cells to IgE
- Induce influx of T cells, monocytes, and granulocytes but not eosinophils
- Augmented production of C-C chemokines (RANTES, MCP-3, and MIP-1α but not eotaxin) on intranasal challenge

IgE and Th2

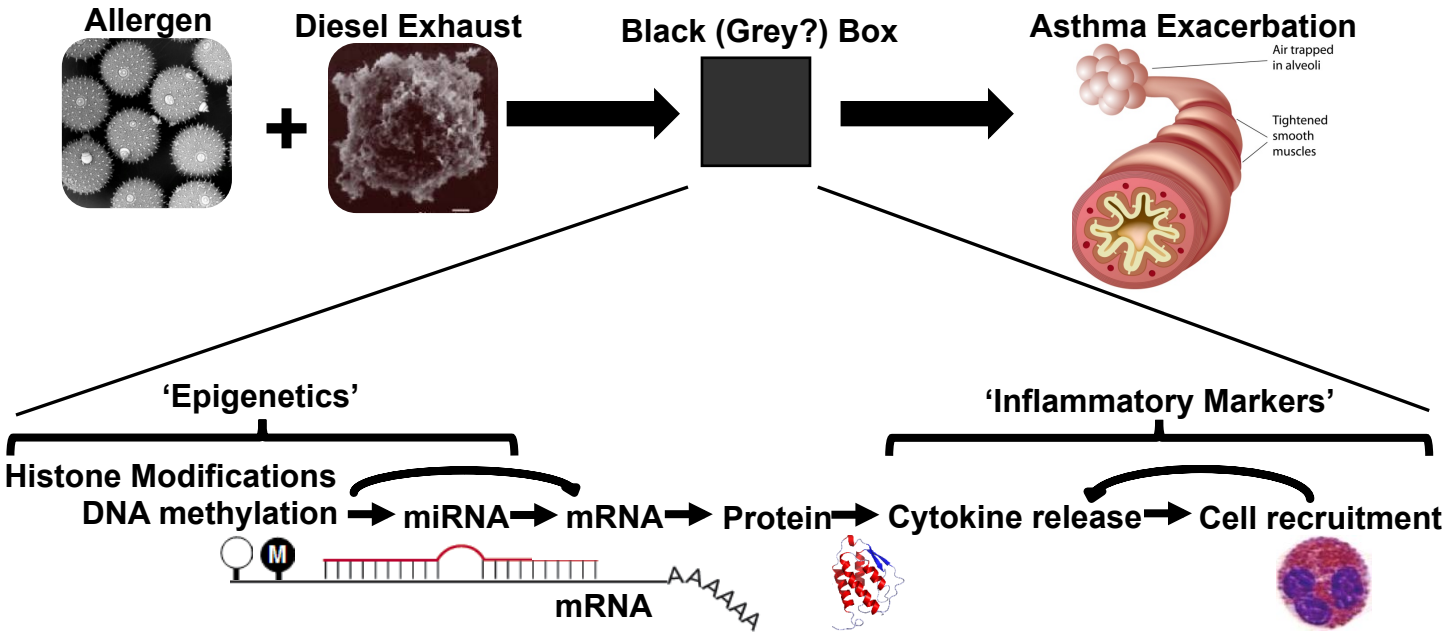
PM plus allergen: ↑ specific IgE in rat **blood** at 4d post-exposure



Liu *et al.*, Tox Sci, 2008 (rats; DE = 1.3 **mg**/m^3 by Cummins engine)

DE → changes in DNA methylation in promoter of IL-4 (↓) and IFN-gamma (↑)

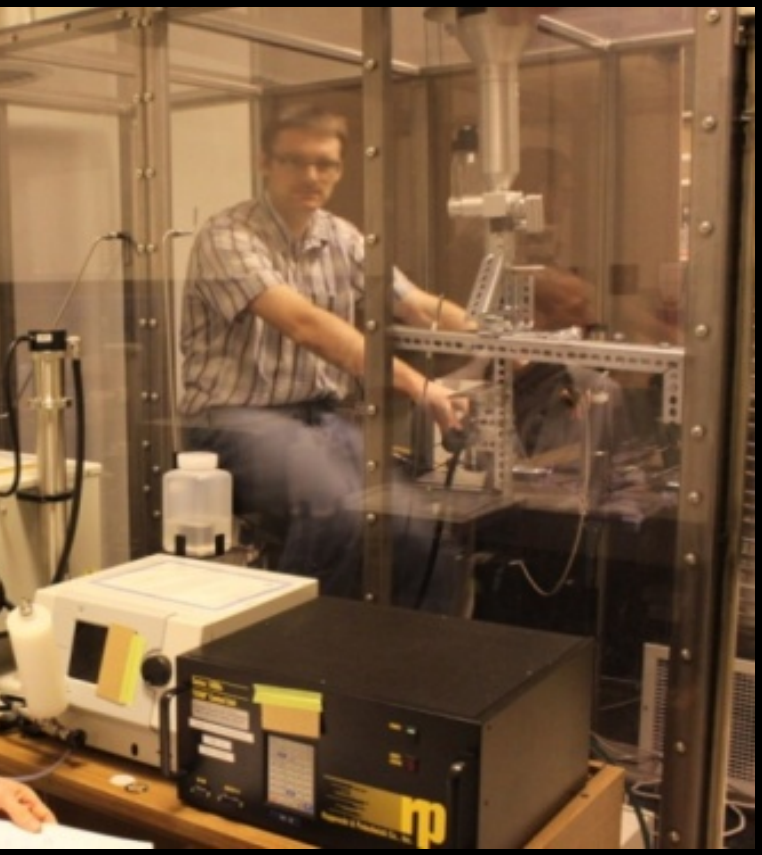
Air Pollution, Allergens and Asthma Exacerbations



The Air Pollution Exposure Laboratory (APEL) for controlled human exposure to diesel exhaust and other inhalants: characterization and comparison to existing facilities

Nicholas Birger[1], Timothy Gould[2], James Stewart[2], Mark R. Miller[3], Timothy Larson[2], and Chris Carlsten[1]

Inhalation Tox 2011 PMID: 21438706



Design allows real-time control over:

- Concentration of particles and gases

  ('smoggy day in Asia')
- Duration of exposure
- Ventilation rate
- Temperature and humidity
- *Innumerable individual characteristics, including genetics (key advantage of crossover design)*
- *Free of endotoxin contamination*

Are diesel exhaust and allergen synergistic in the lung?

Using realistic diesel exhaust exposure

18 volunteers sensitized to Timothy grass, birch or house dust mite

Diesel exhaust (300 ug $PM_{2.5}/m^3$) vs. air

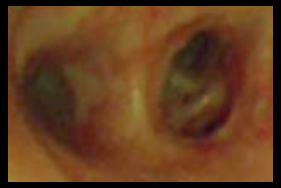
(randomized *crossover*)

followed by segmental allergen challenge

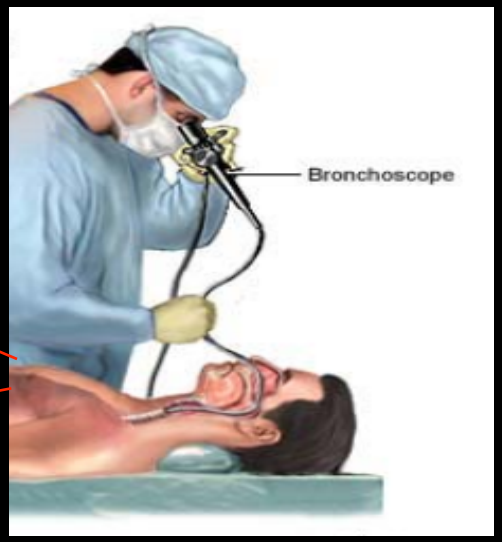
Right lung

SALINE

Left lung



ALLERGEN

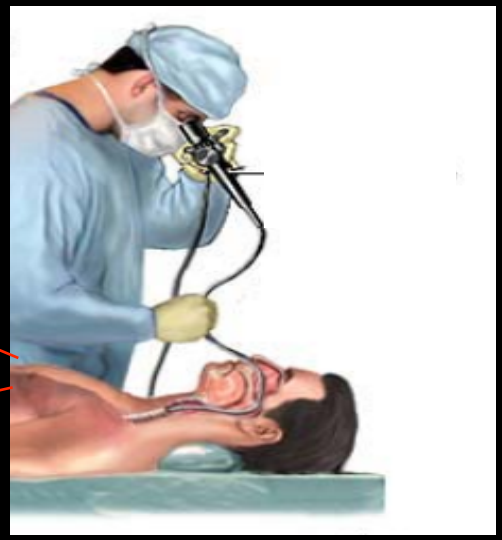


Carlsten *Thorax 2015* PMID: 26574583

48 hours later. . .

Right lung

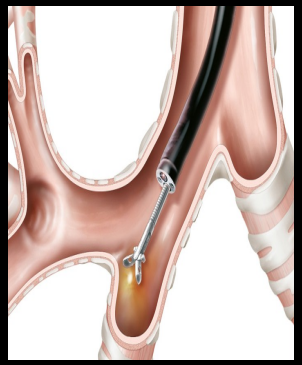
Left lung



Wash

biopsy, brush

>95% epithelial cells



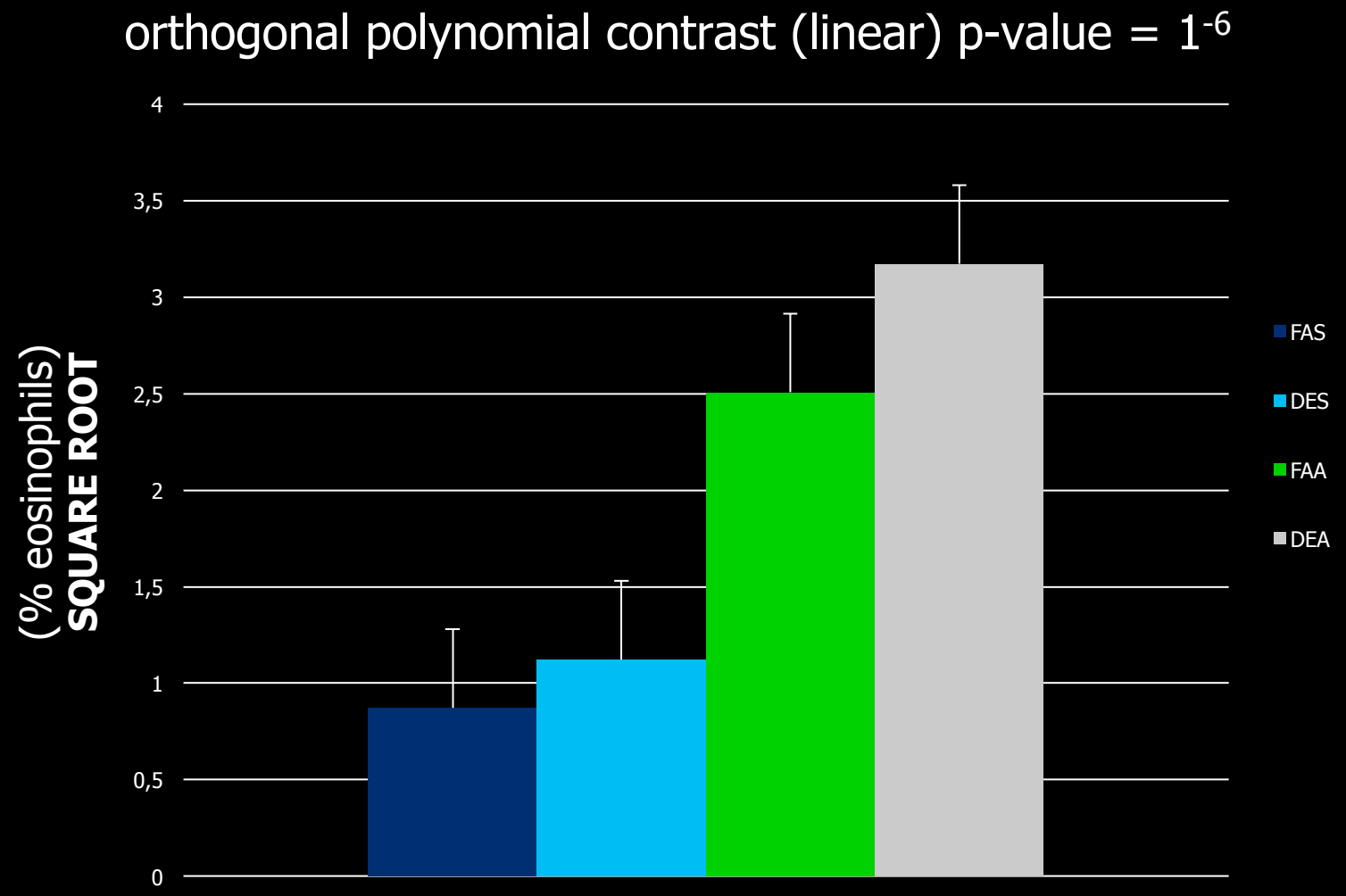
4 weeks later. . .

Repeated identically but for initial exposure (DE or filtered air)

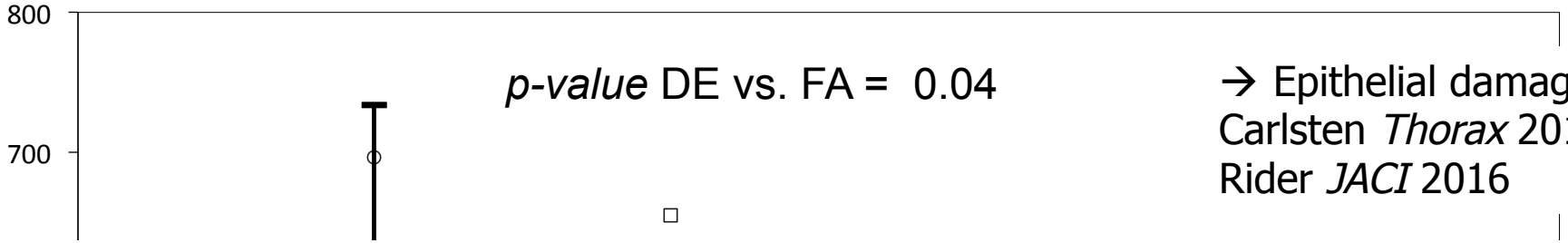
Carlsten *Thorax 2015* PMID: 26574583

Carlsten, Thorax 2015 PMID: 26574583

BAL 48-hr post-exposure: % eosinophils



Biagioni, Sin, Carlsten *Clinical and Experimental Allergy* 2016 PMID: 27018153

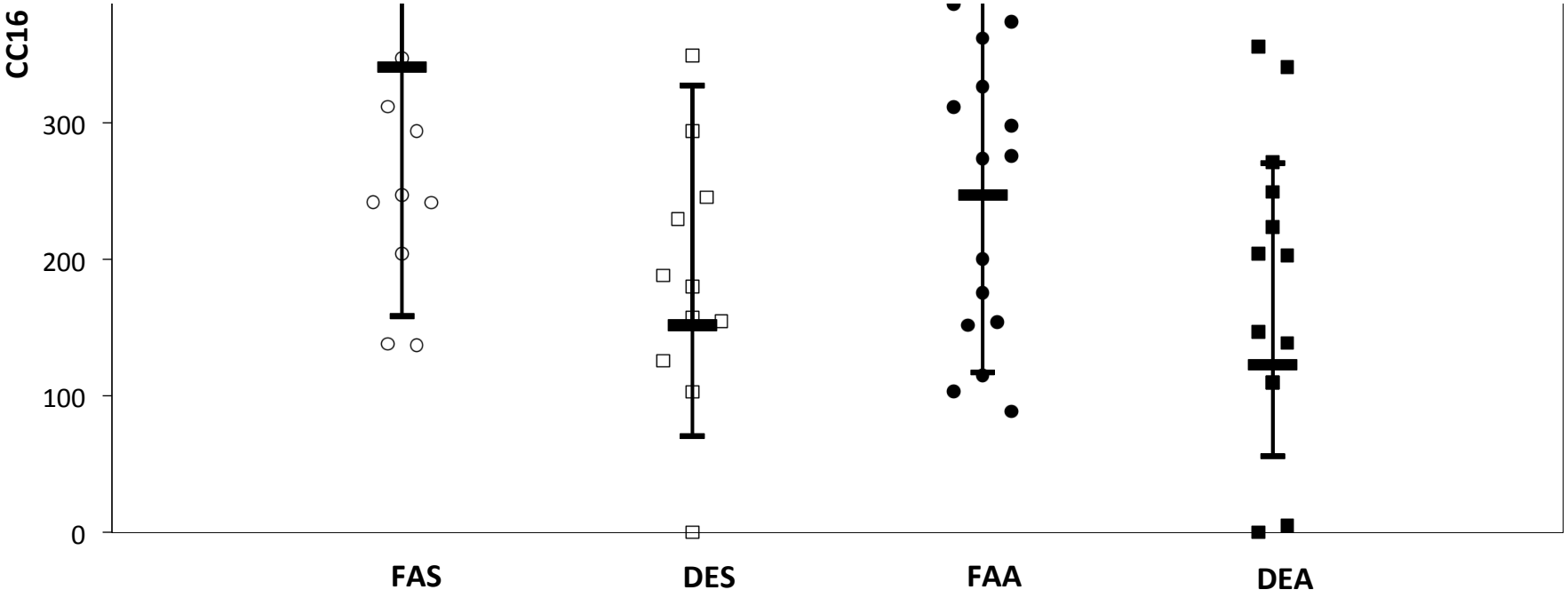


→ Epithelial damage
Carlsten *Thorax* 2016
Rider *JACI* 2016

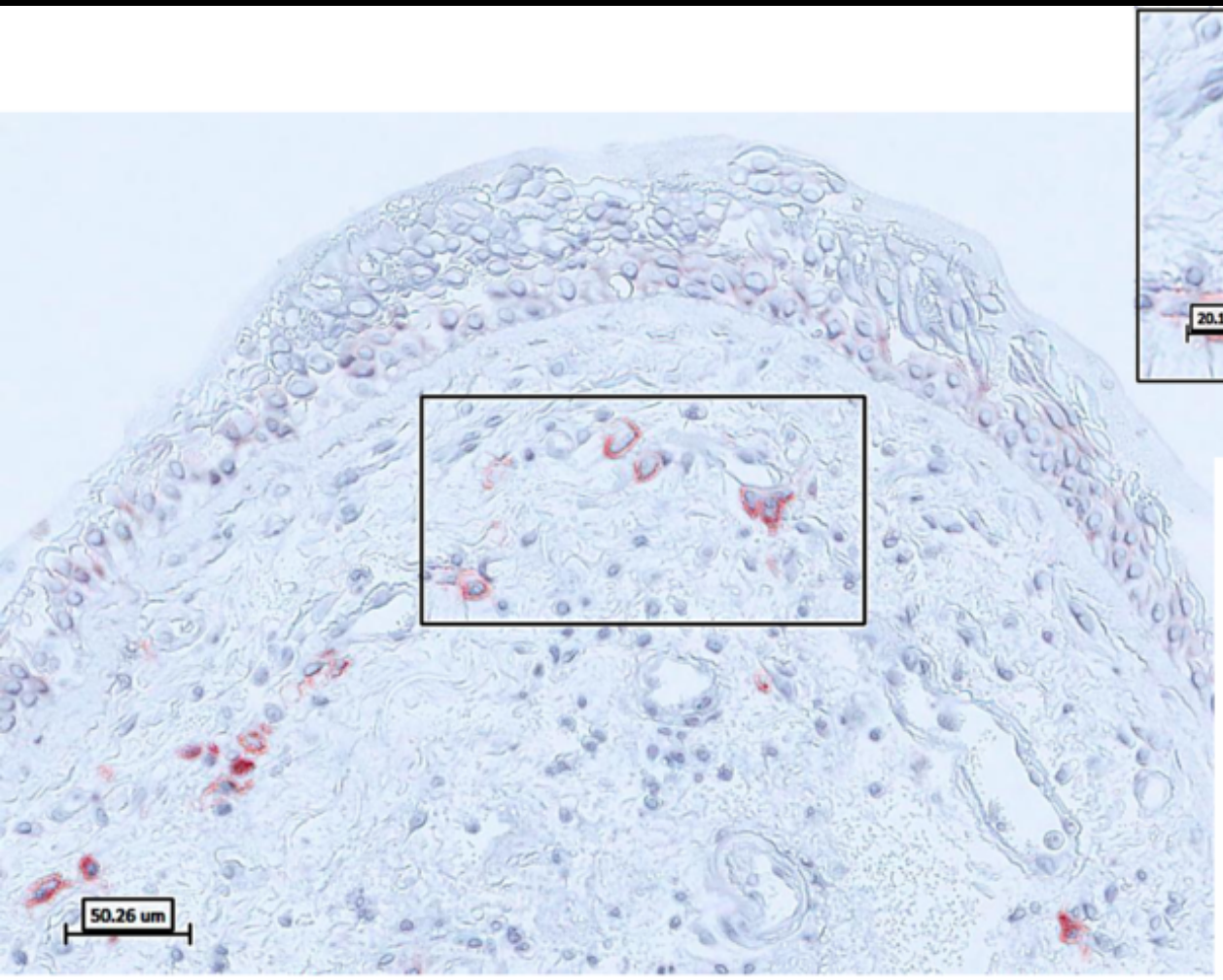
Club Cell Protein 16 and Disease Progression in Chronic Obstructive Pulmonary Disease

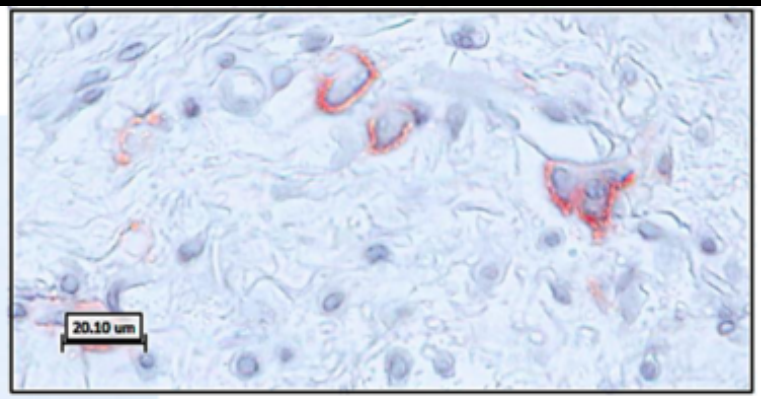
Am J Respir Crit Care Med Vol 188, Iss. 12, pp 1413–1419, Dec 15, 2013

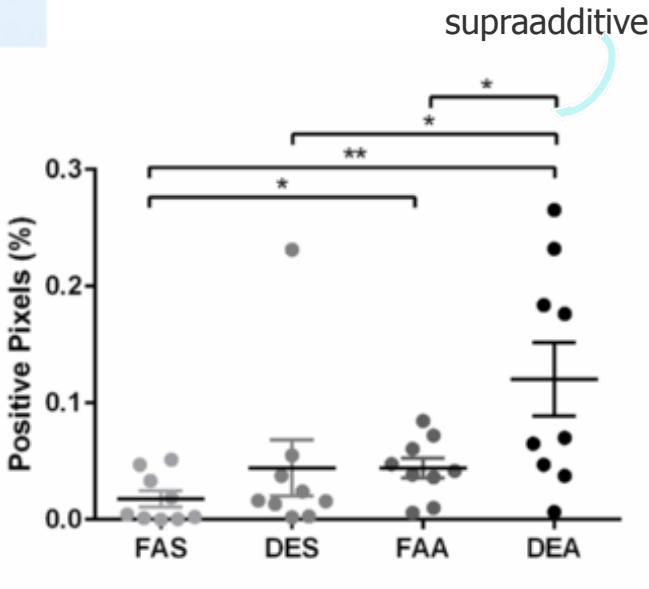
Hye Yun Park[1,2], Andrew Churg[3], Joanne L. Wright[3], Yuexin Li[1], Sheena Tam[1], S. F. Paul Man[1,4], Donald Tashkin[5], Robert A. Wise[6], John E. Connett[7], and Don D. Sin[1,4]



Endobronchial biopsy submucosal CD138 (plasma cells)



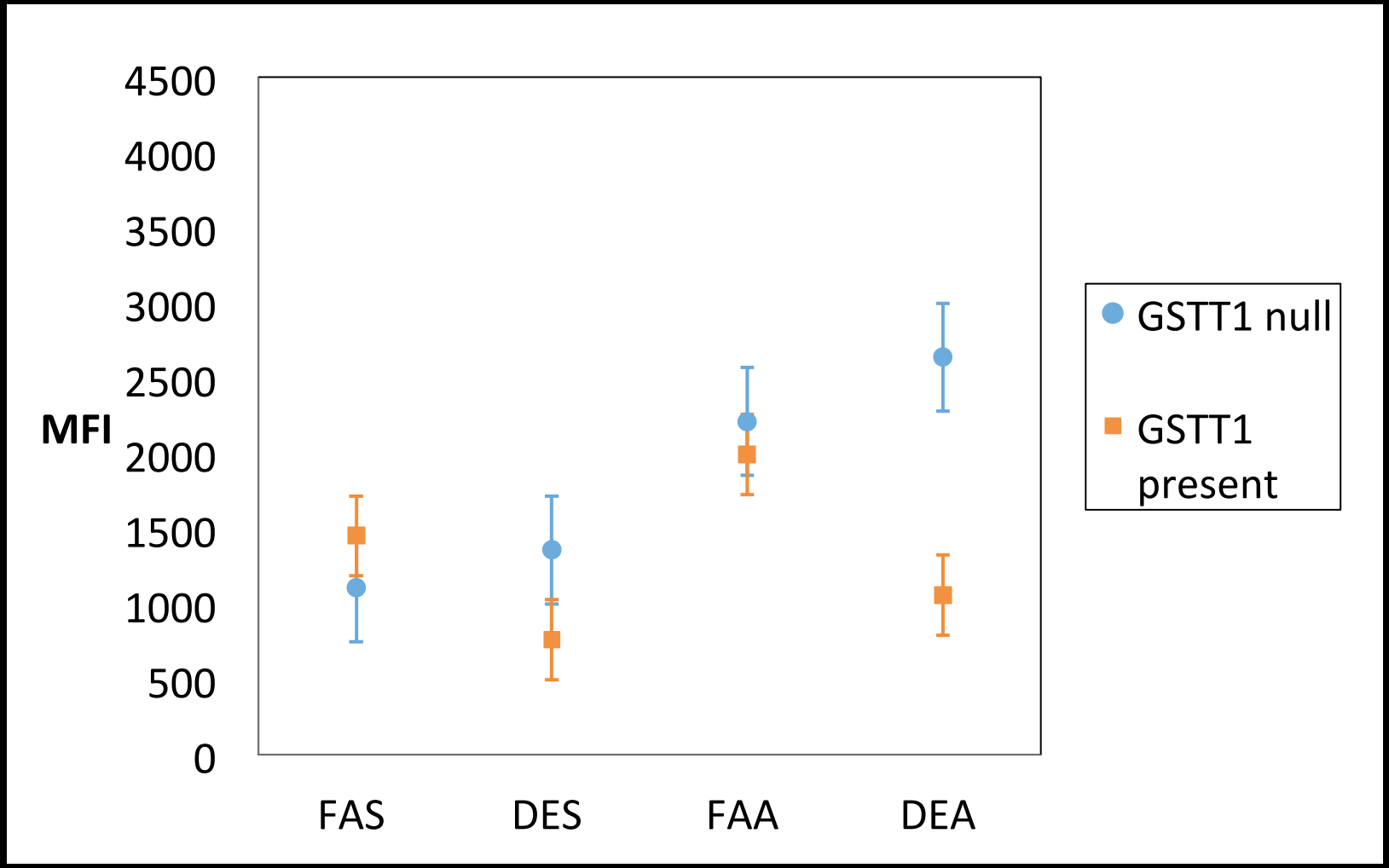




Hosseini *et al* Particle Fibre Tox 2016 PMID: 26758251

GENE-EXPOSURE-EXPOSURE INTERACTION

GSTT1-by-exposure interaction on activation of cytotoxic T cells* in lung lavage

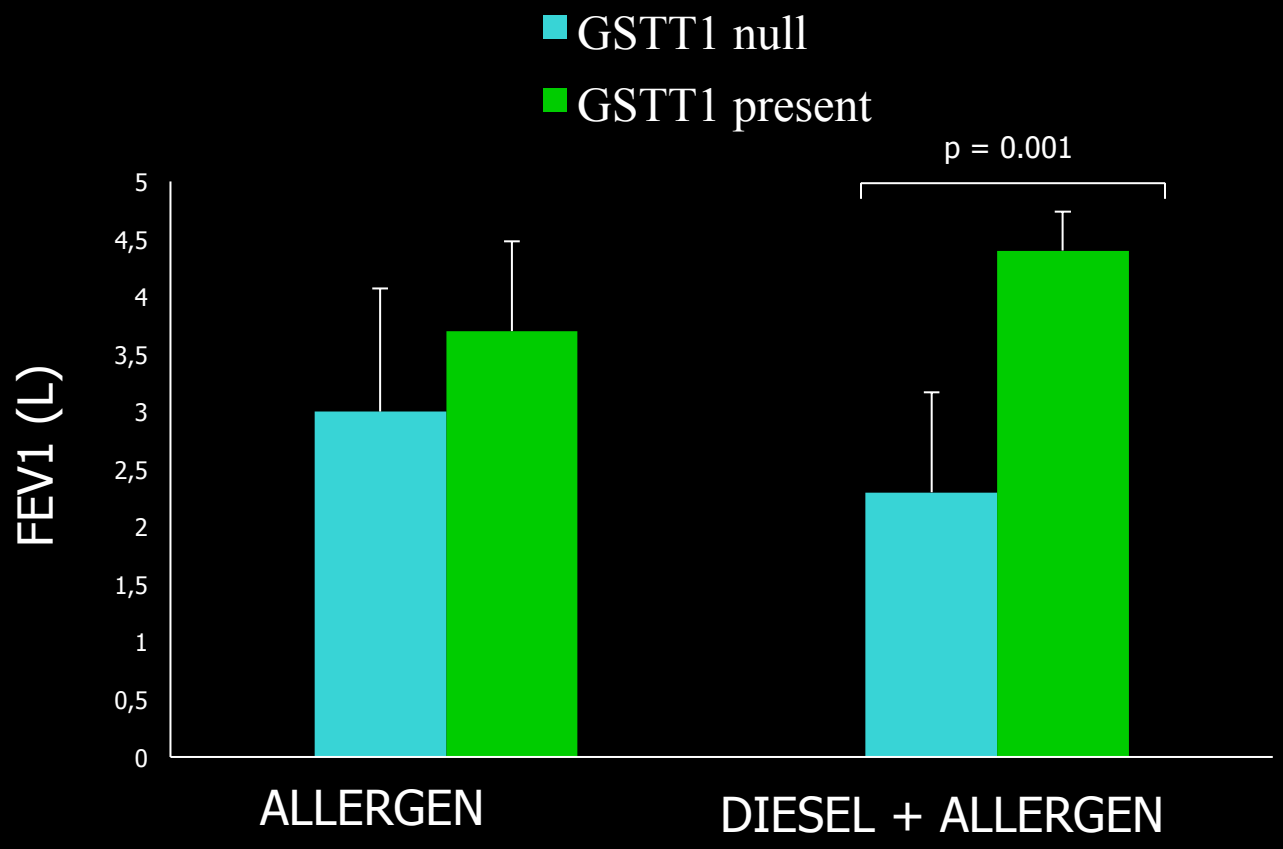


*CD3+/CD4-/CD8+/CD19-/CD45+/CD69+

Carlsten *Thorax 2015* PMID: 26574583

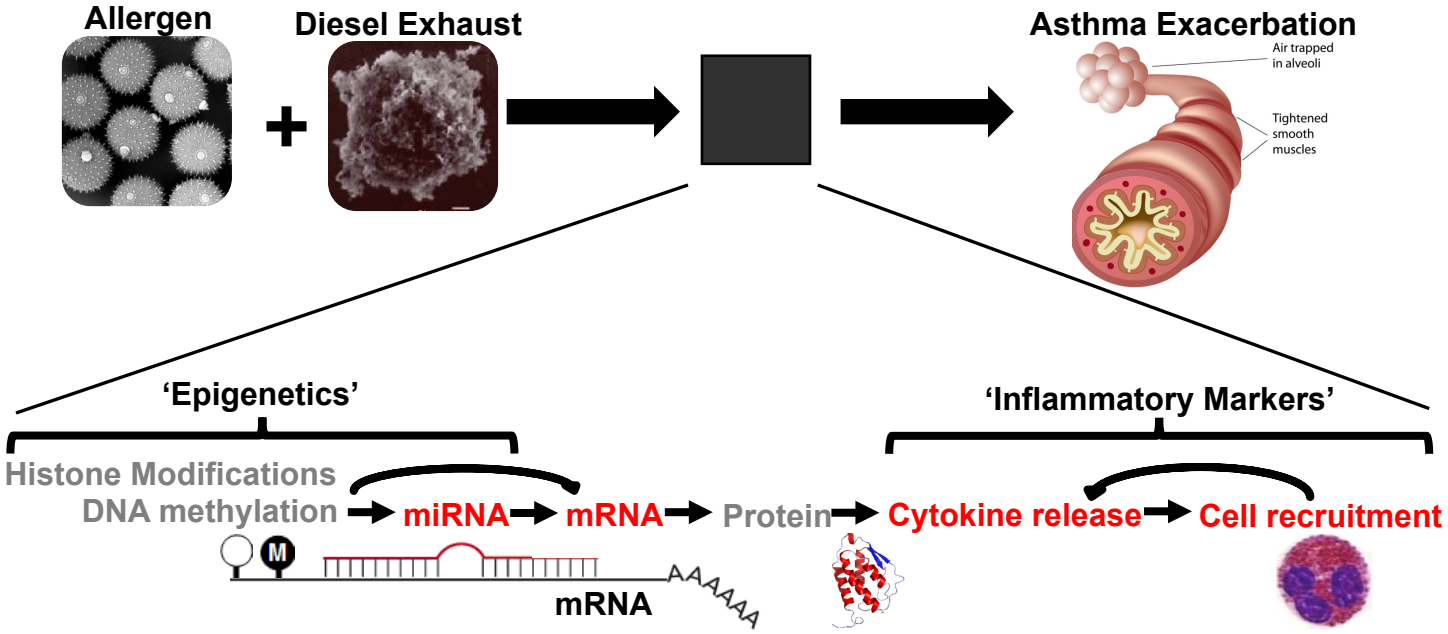
Effect of combined exposures *and* genotype

2hr after allergen exposure, airflow is reduced in GSTT1 null, but only if also exposed to DE



Xin Zhang, *FRBM* 2016

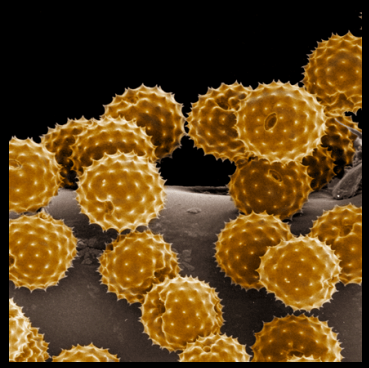
Air Pollution and Asthma Exacerbations



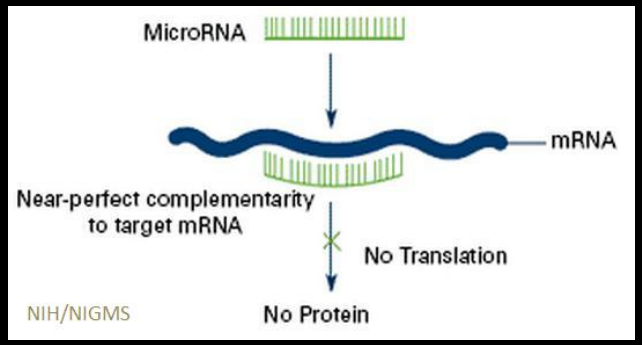
Hypothesis: Acute exposure to diesel exhaust and/or allergen alters expression of miRNA and genes, with effects on inflammatory markers, associated with asthma.

Effect of combined exposures on epithelial epigenetics

Δ miRNA, ΔDNA methylation



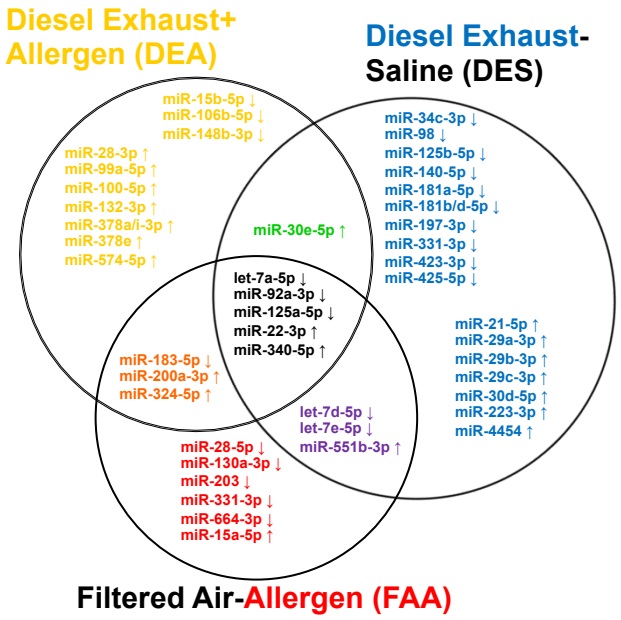
Rider *et al*
JACI 2016

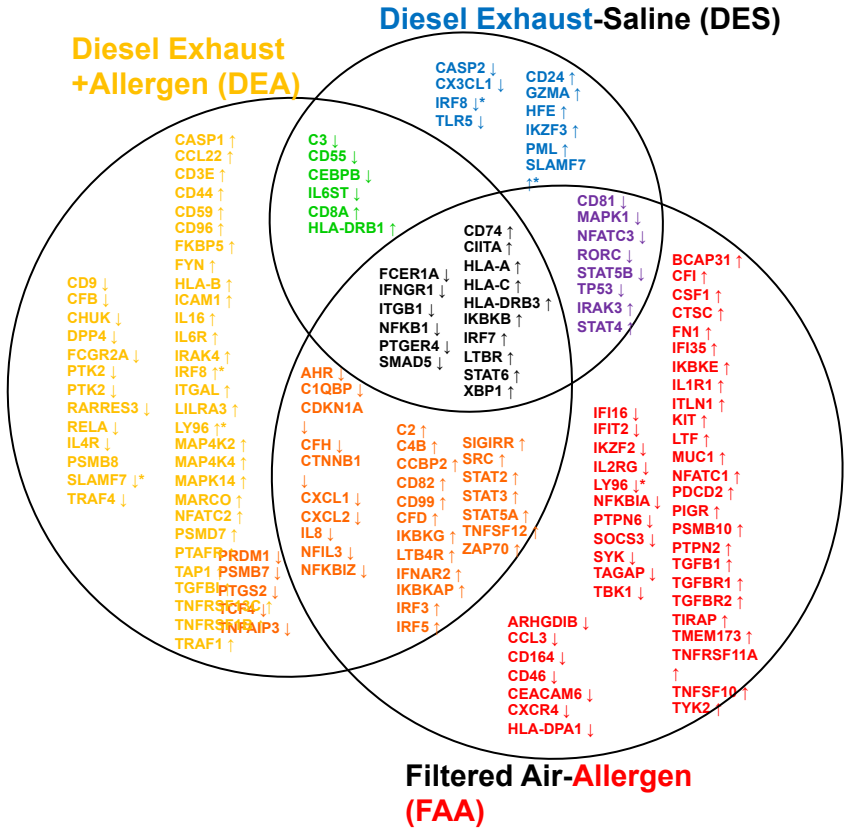


Clifford *et al*
JACI 2016

Rider *et al*
JACI 2016

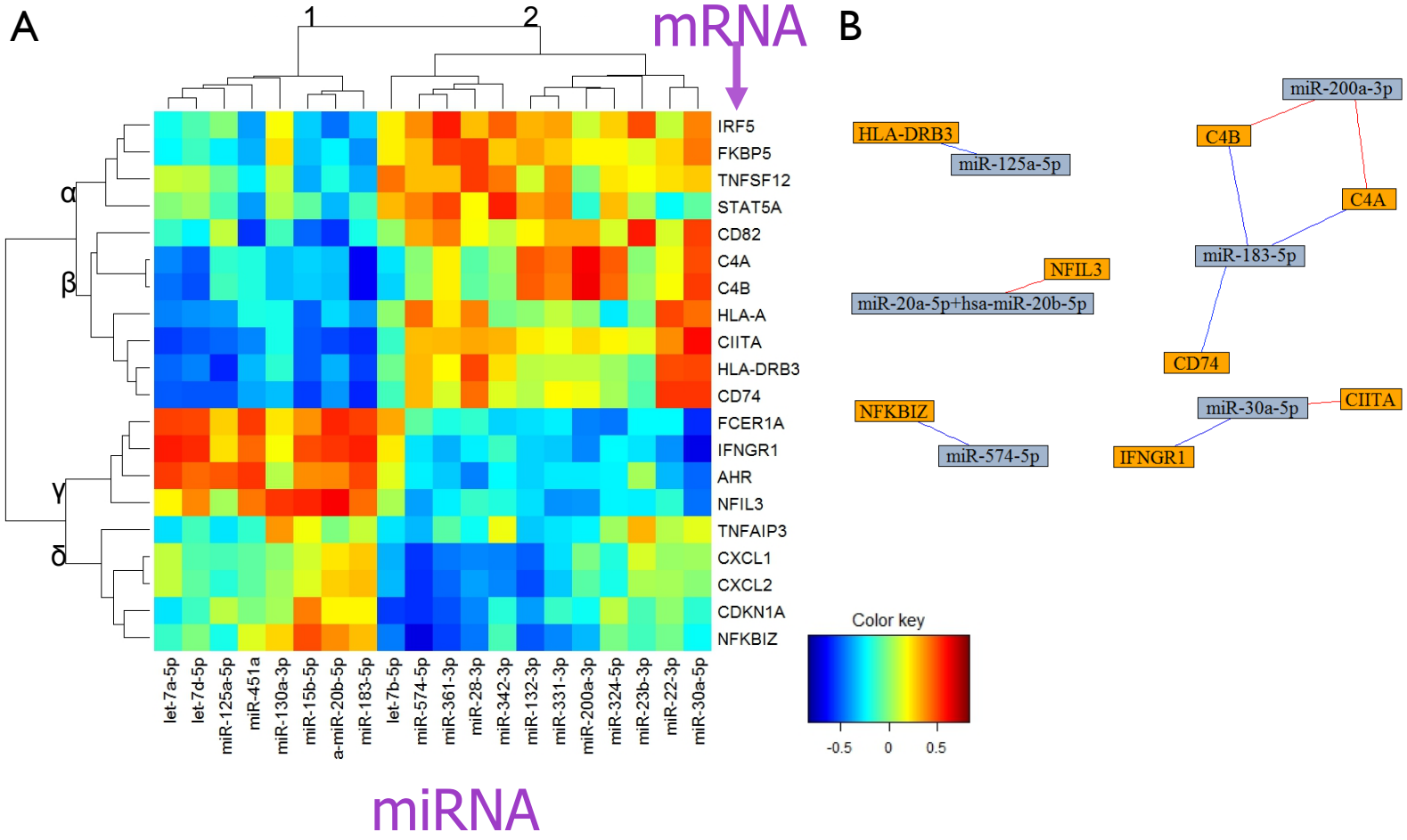
miRNA and mRNA response
(to various conditions, relative to control)





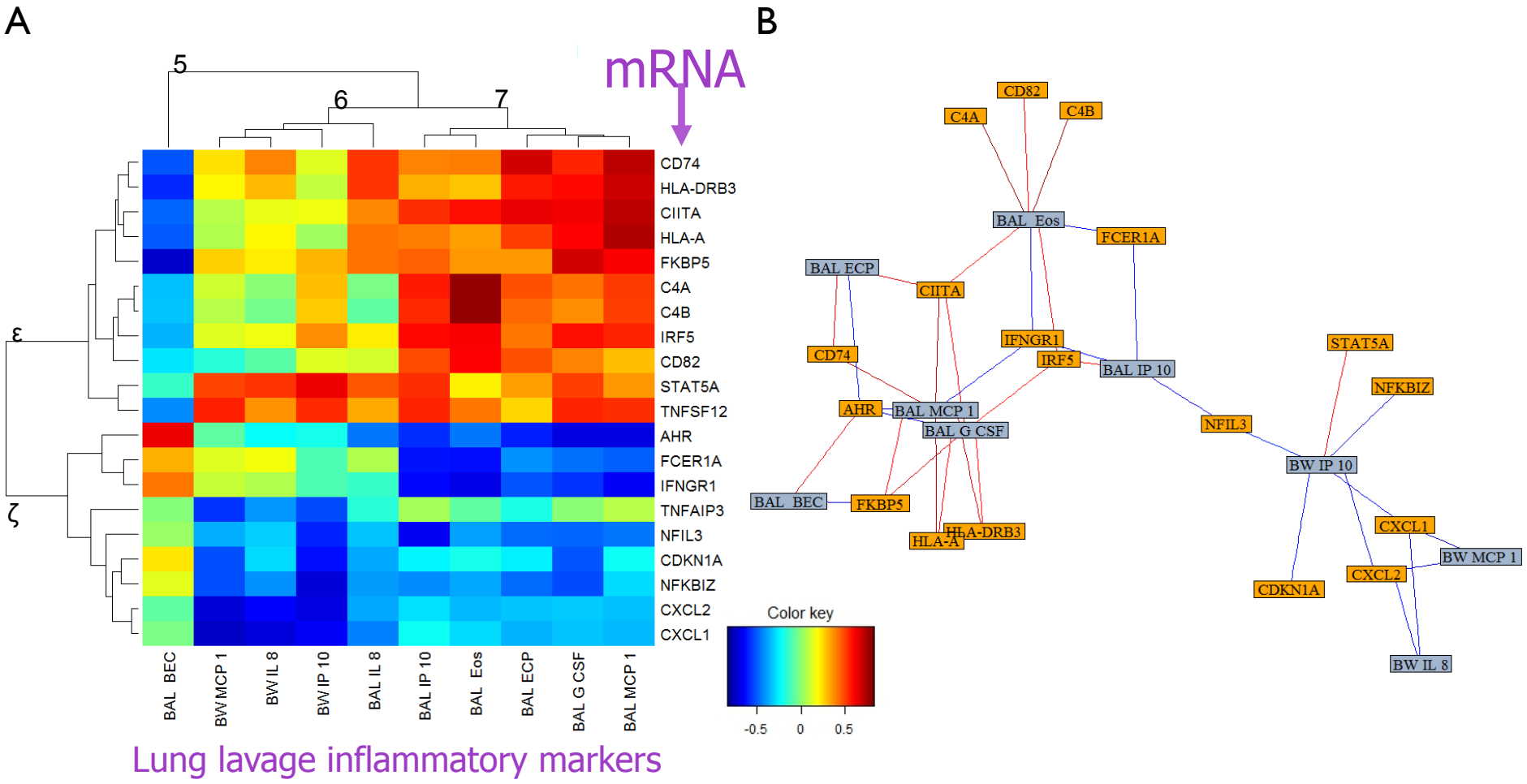
Nanostring nCounter™ Human miRNA v2 (includes 800 miRNAs) and Human Immunology v1 (includes 511 mRNAs)

Correlations between miRNAs and gene expression



miRNA → gene expression → inflammation

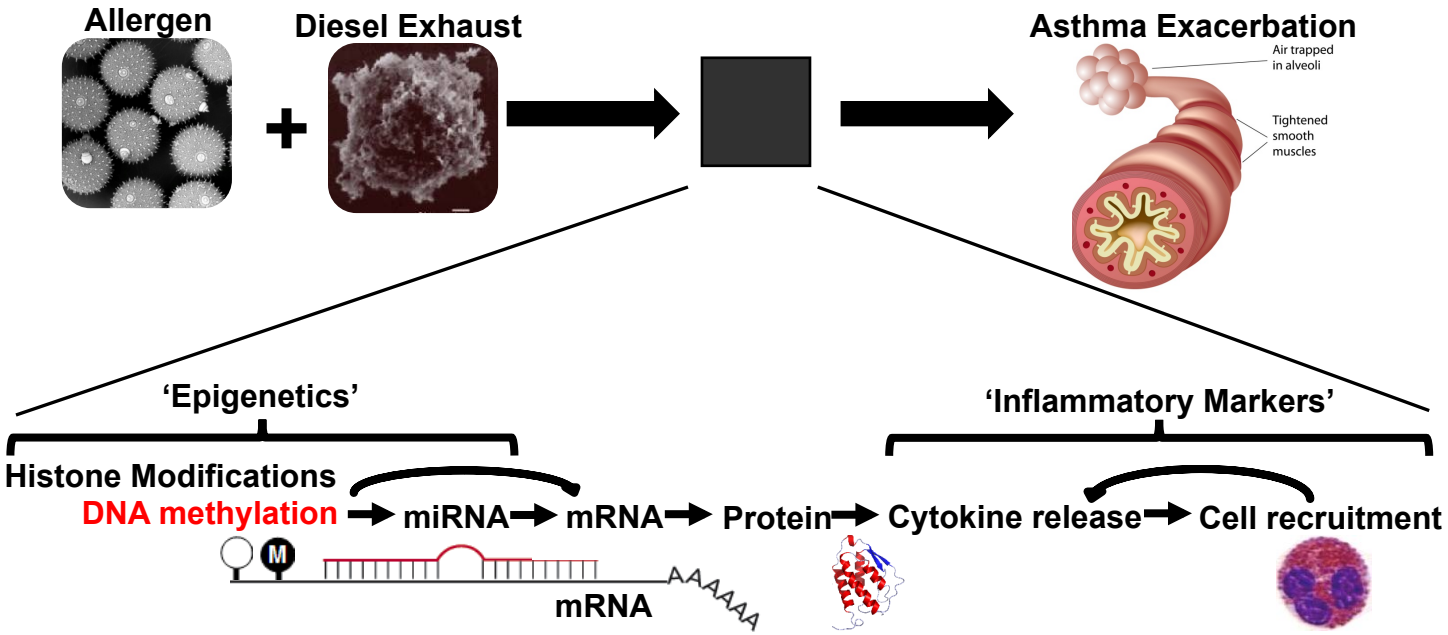
Correlations between gene expression and inflammatory markers



Lung lavage inflammatory markers

miRNA → gene expression → inflammation

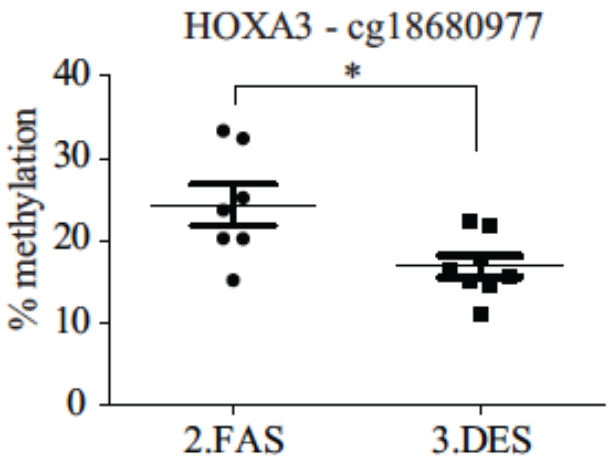
Air Pollution and Asthma Exacerbations

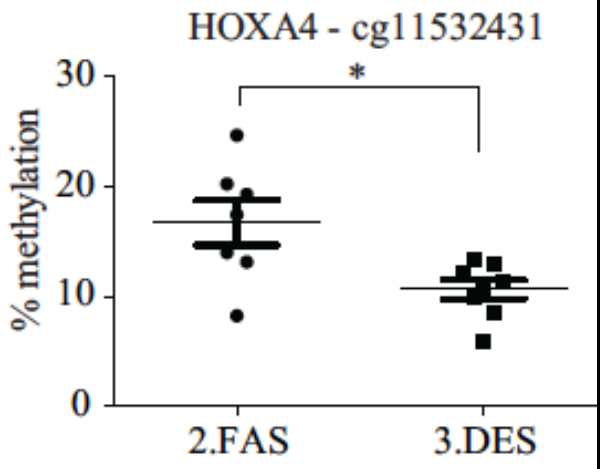


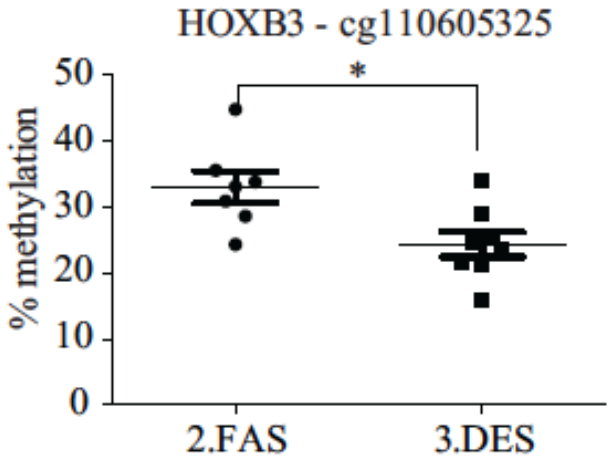
Top methylation changes in **airway epithelium** after acute DE exposure

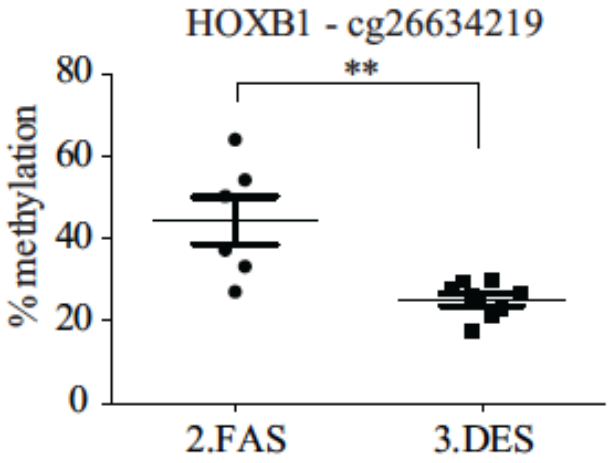
Demethylation of *Homeobox* genes

Encode highly conserved cell differentiation transcription factors







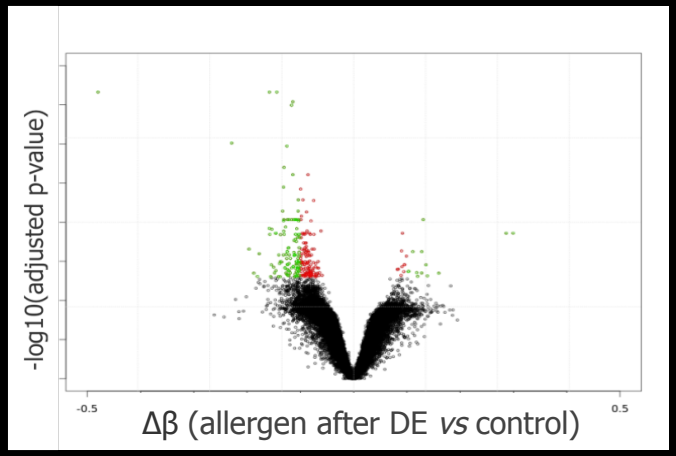


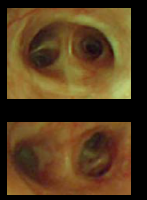
Clifford *et al*, *JACI* 2016

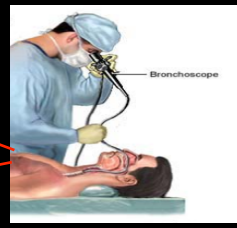
EXPOSURE-EXPOSURE-EPIGENETIC INTERACTION

Clifford *et al*, JACI 2016

DNAme (Illumina 450K)





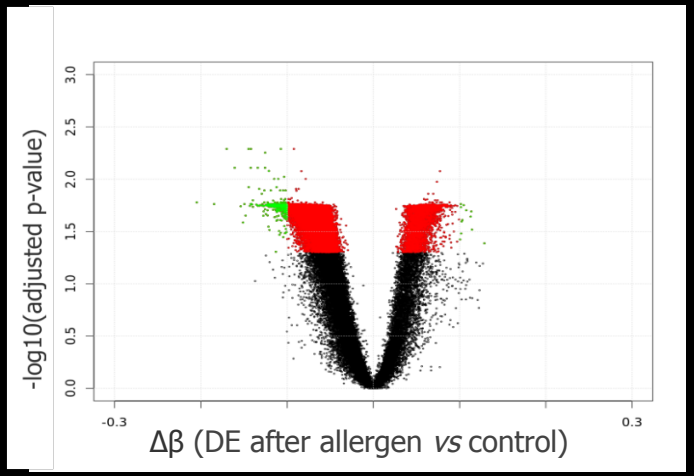


4 wks

DE → allergen

(129 green hits)

DNAme dependent on order of exposures



4 wks

allergen → DE

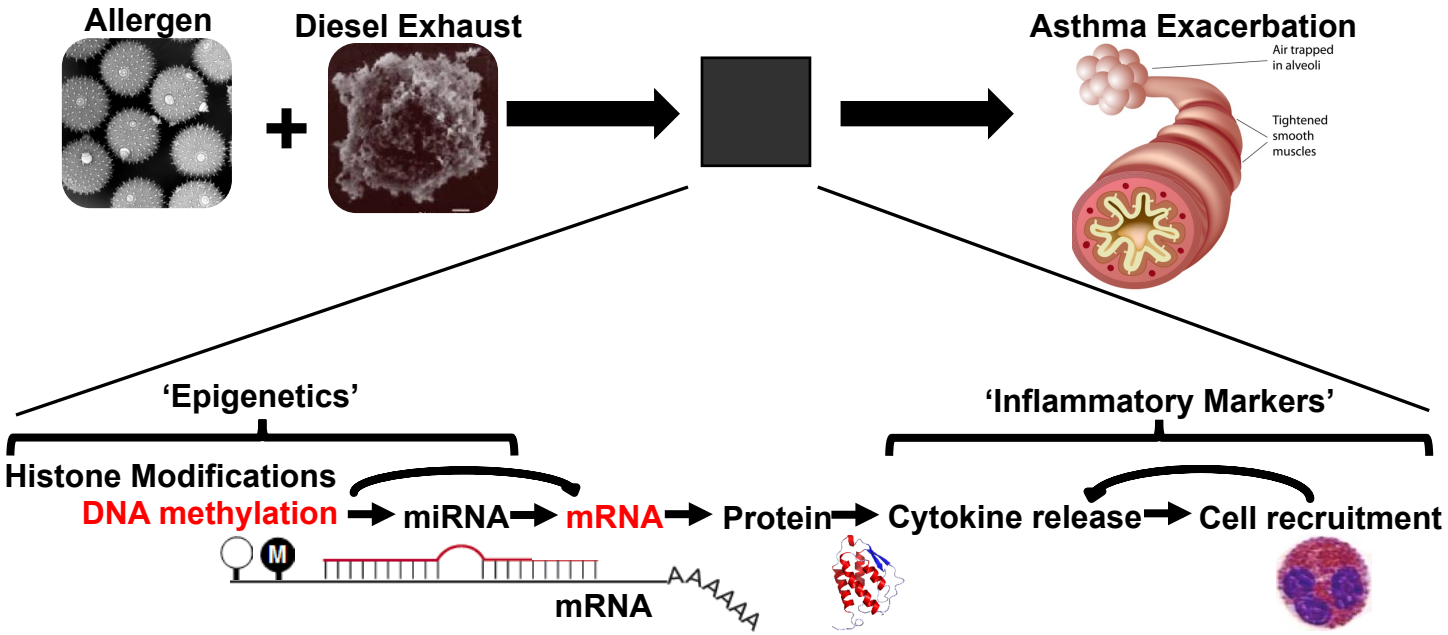
(535 green hits)

Significant probes (FDR-corrected p-values < 0.05) are RED

Probes significant *and* with a Δβ >0.1 (10% methylation change) are GREEN

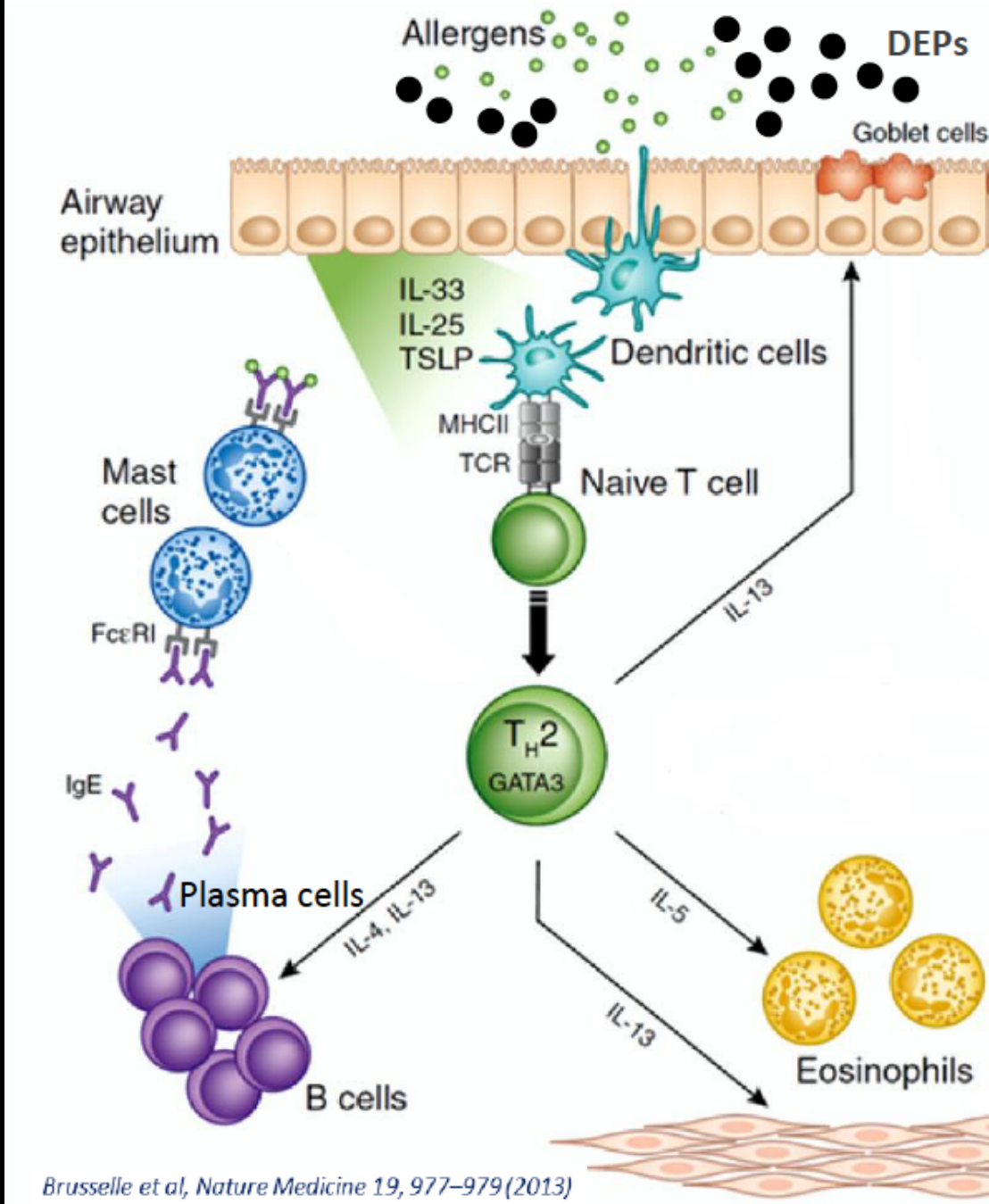
Air Pollution, Allergen and Asthma Exacerbations

Next steps – more integrative 'omics



Possible mechanisms 'where?'

- Cells within airway lumen
- Epithelium (+/- damage)
- Subepithelium
- Circulation



How does exposure to gas, dust and fumes enhance sensitization and asthma?

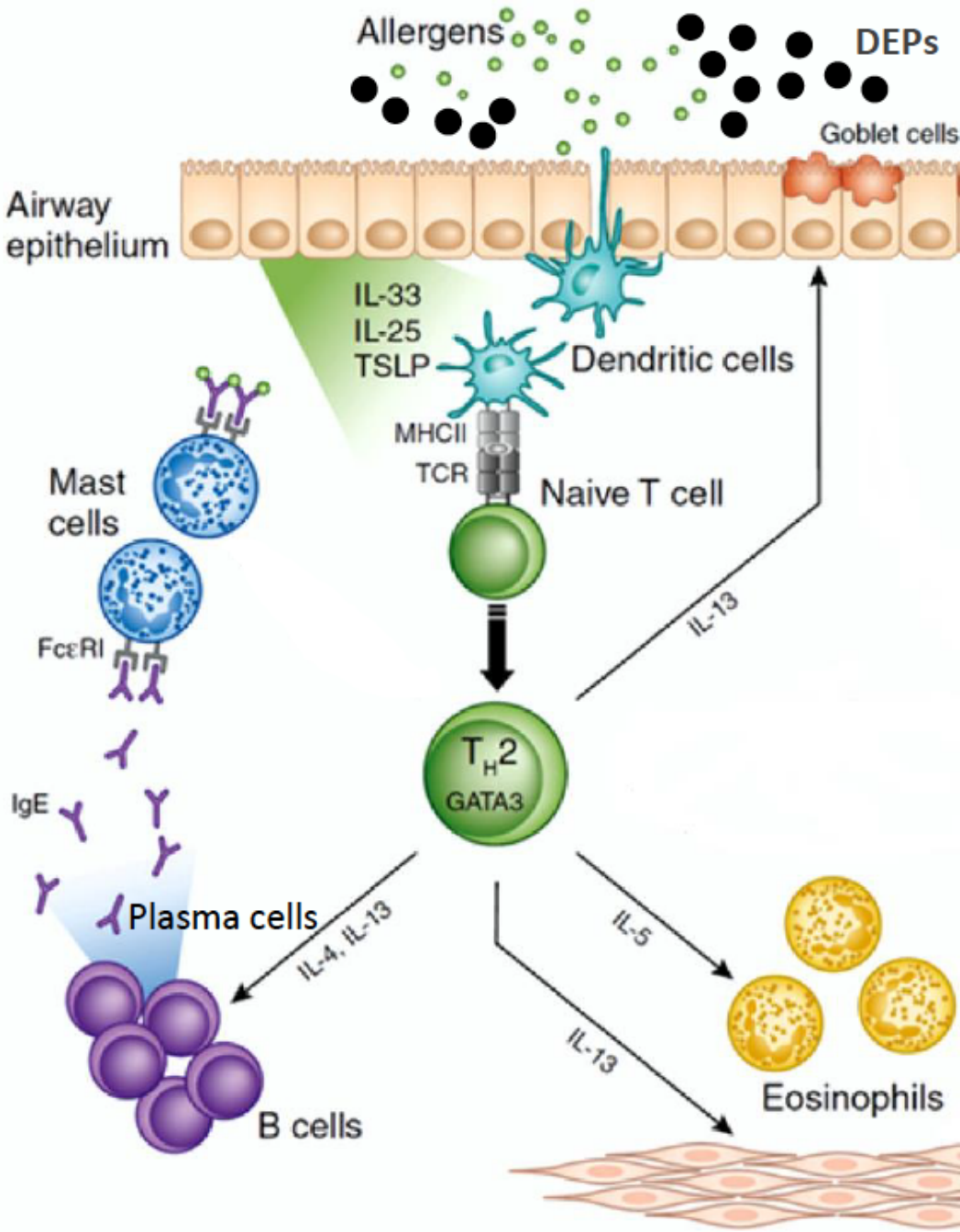
Possible mechanisms 'how?'

Classic cells and mediators

Novel mediators

- Transcriptome
- Proteome (A2876 Monday am)
- Epigenome

Modifying factors (gene variants)



APEL Air Pollution Exposure Laboratory

UBC Chan-Yeung Centre for Occupational and Environmental Respiratory Disease

Understanding exposure effects – linking research to public health

pollutionlab.wordpress.com
@pollutionlab

Our Mission:

To gain new understanding of the mechanisms involved in occupational and environmental lung disease through laboratory and clinical research, and to translate this knowledge into improved diagnostic, therapeutic, and preventative tools for the benefit of public health

We welcome highly-motivated and team-oriented trainees

WorkSafeBC

Vancouver Coastal Health Research Institute

Canada Foundation for Innovation

AllerGen

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

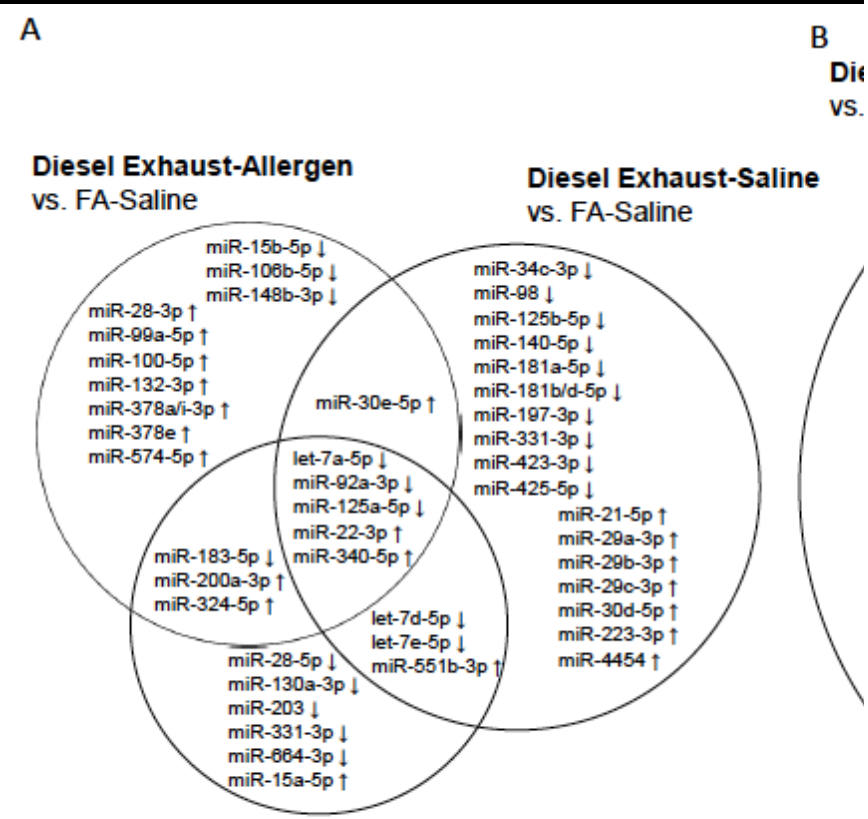
Institute for HEART+LUNG Health
Strong beats. Clear breaths. Full lives.

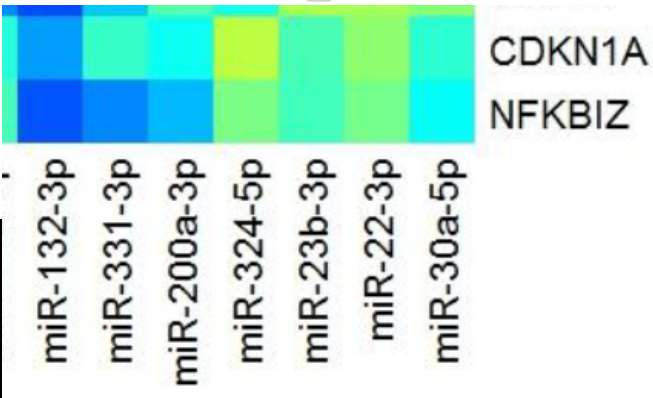
THE LUNG ASSOCIATION™ British Columbia

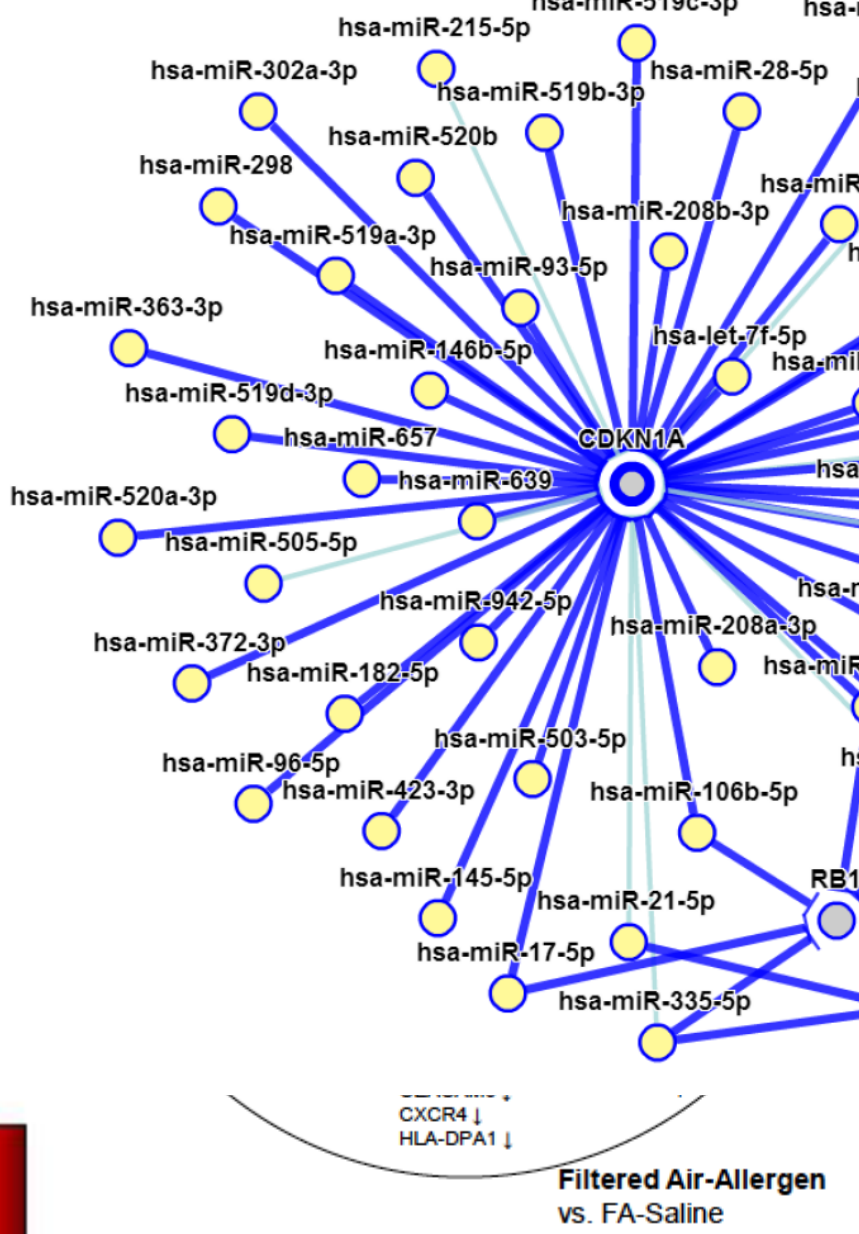
MICHAEL SMITH FOUNDATION FOR HEALTH RESEARCH
Discover. Connect. Engage.

CRRN RCRR
Canadian Respiratory Research Network / Réseau canadien de recherche respiratoire

miR-132-3p ↑







CDKN1A ↓

APEL data compared with DDS

Focusing on DE+Ag v. Ag only

Diaz-Sanchez et al **Mouse models** (2002)	APEL **Our prelim human lung data**
→ DE: up to 2000 ug/m^3 for 1hr daily for 10 days	→ DE: 300 ug/m^3 for 2 hrs
→ 19-fold increase in specific IgE	→ 1.6-fold increase in specific IgE
→ No sig. change in BAL eos %	→ 4x increase in BAL eos%

APEL data compared with DDS

Focusing on DE+Ag v. Ag only

Diaz-Sanchez et al **Human nasal models** (1997)	APEL **Our prelim human lung data**
→ 0.3mg DEP + 200U Ag	→ DE: 300 ug/m^3 for 2 hrs + SA
→ No change in total IgE levels	→ No diff in total IgE
→ 6x increase in specific IgE @ 1d-post; 16x increase 4d post	→ 1.6x increase in specific IgE
→ 7x increase in IL-5 mRNA IL-5	→ 2x increase in IL-5
→ Did not look at Eos	→ 4x increase in Eos

Possible mechanisms
'how?' (2)

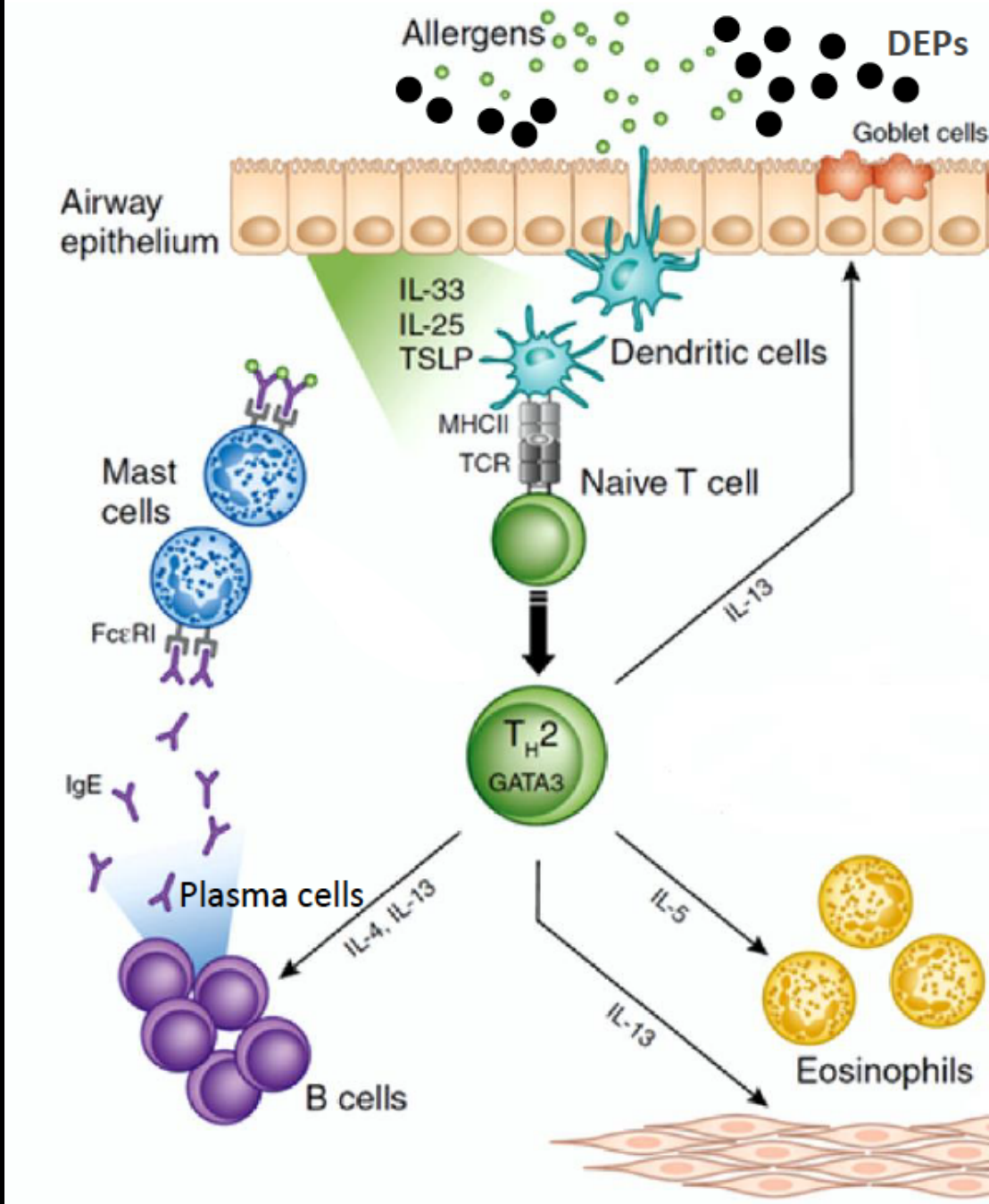
Inhalants directly pass into subepithelium

Epithelial damage (increase access to subepithelium)

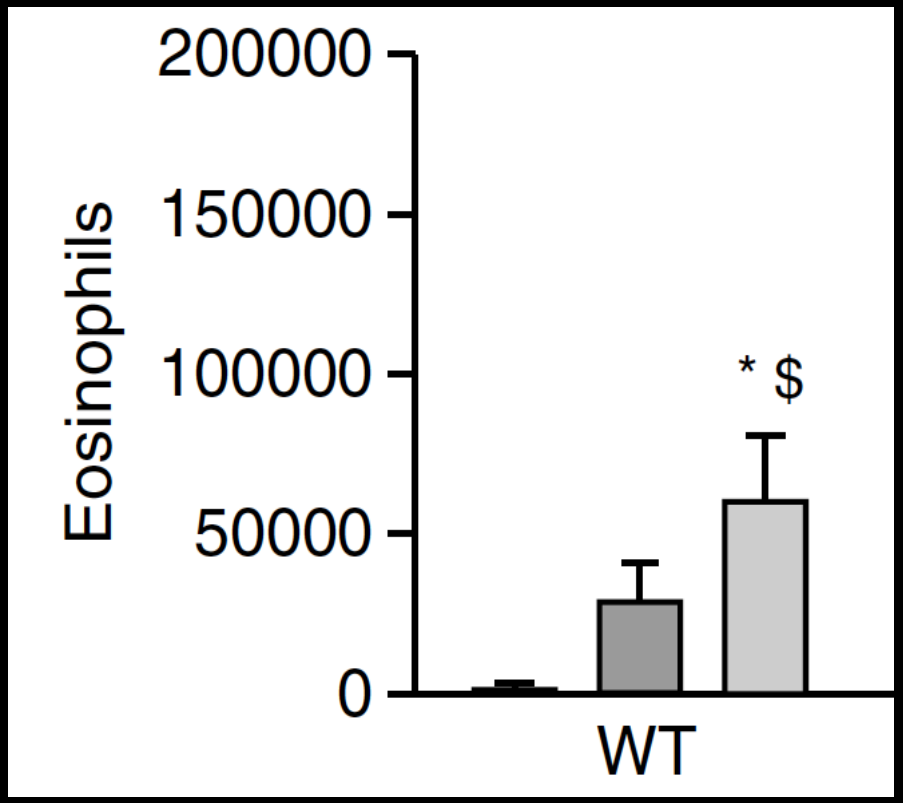
Innate immunity

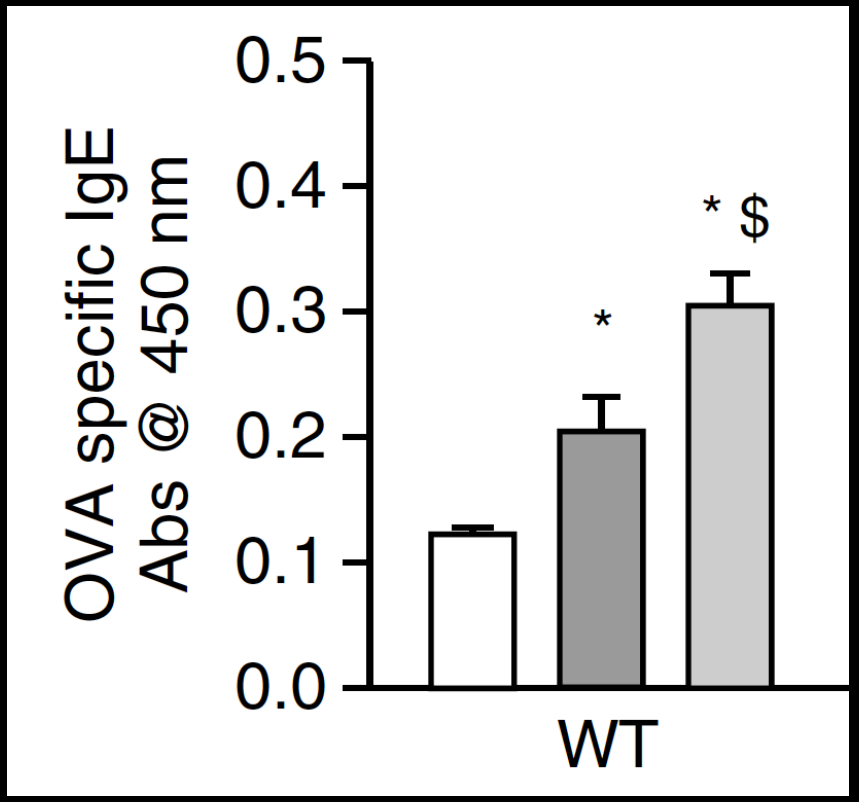
Adaptive immunity

Neuronal pathways



In mice, intranasal PM + OVA
⬆ BAL Eos and specific IgE in **peripheral blood**





Saline OVA OVA/PM_{10}

Hirota *et al.*, AJRCMB 2015 (WT = C57BL/6 mice)

NOTE: Whitekus (Diaz-Sanchez) 2002 found **no increase** in airway eos

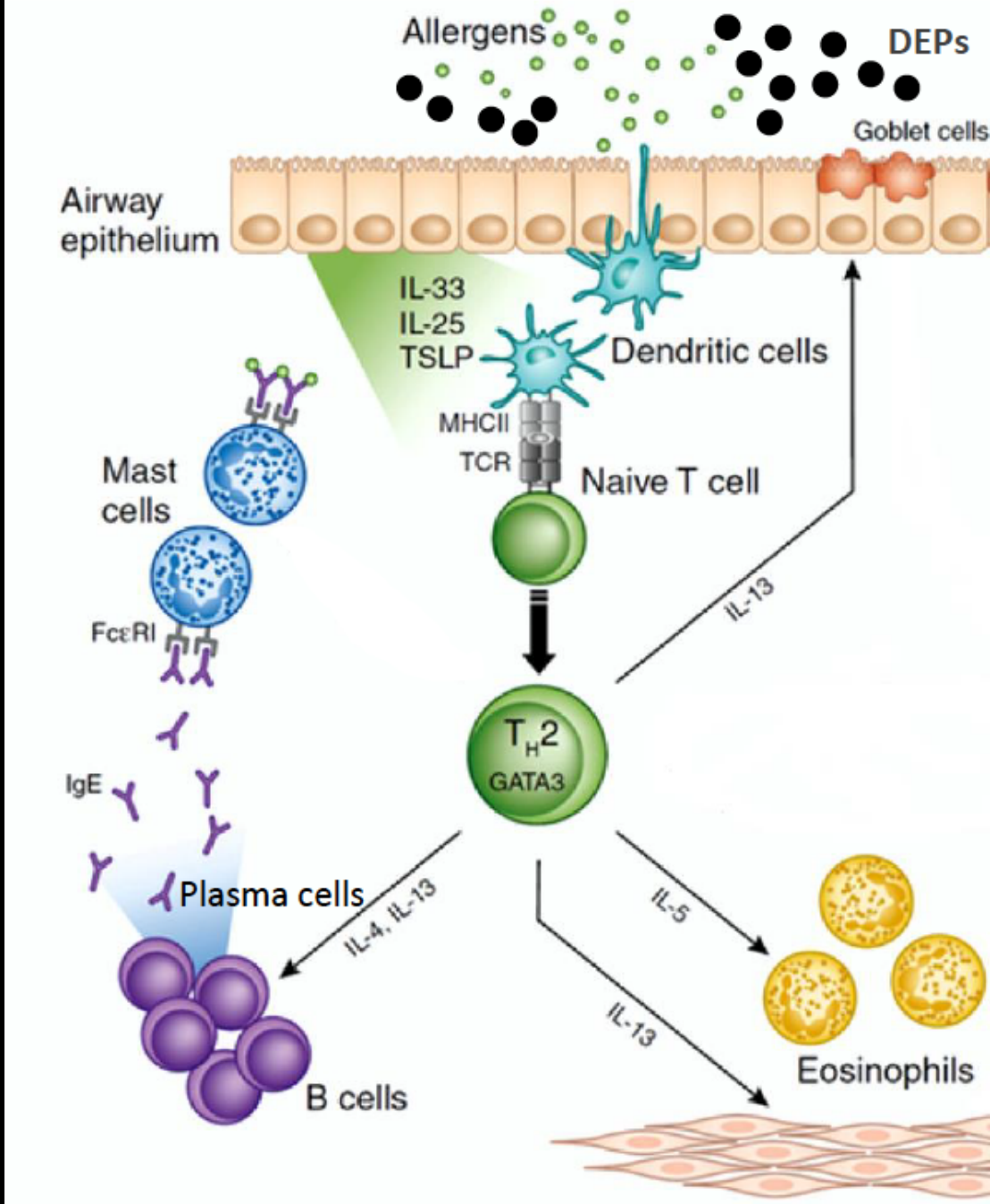
Possible mechanisms

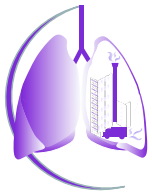
'how?' (1)

In parallel
or
Sequential

Additive
or
Synergistic

Acute
or
Chronic





UBC Chan-Yeung Centre for Occupational and Environmental Respiratory Disease

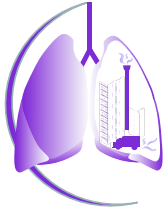
Understanding exposure effects – linking research to public health

APEL Air Pollution Exposure Laboratory

Inhalation of diesel exhaust and allergen alters human bronchial epithelium DNA methylation

JACI 2016

Rachel Clifford
Assistant Professor
University of Nottingham



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Understanding exposure effects – linking research to public health

APEL Air Pollution Exposure Laboratory

MultiOmics of diesel and allergen inhalation *JACI* 2016

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