Group 2 Innate Lymphoid Cells (ILC2s) as Mediators of Air Pollutant-induced Asthma and Rhinitis

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Outline

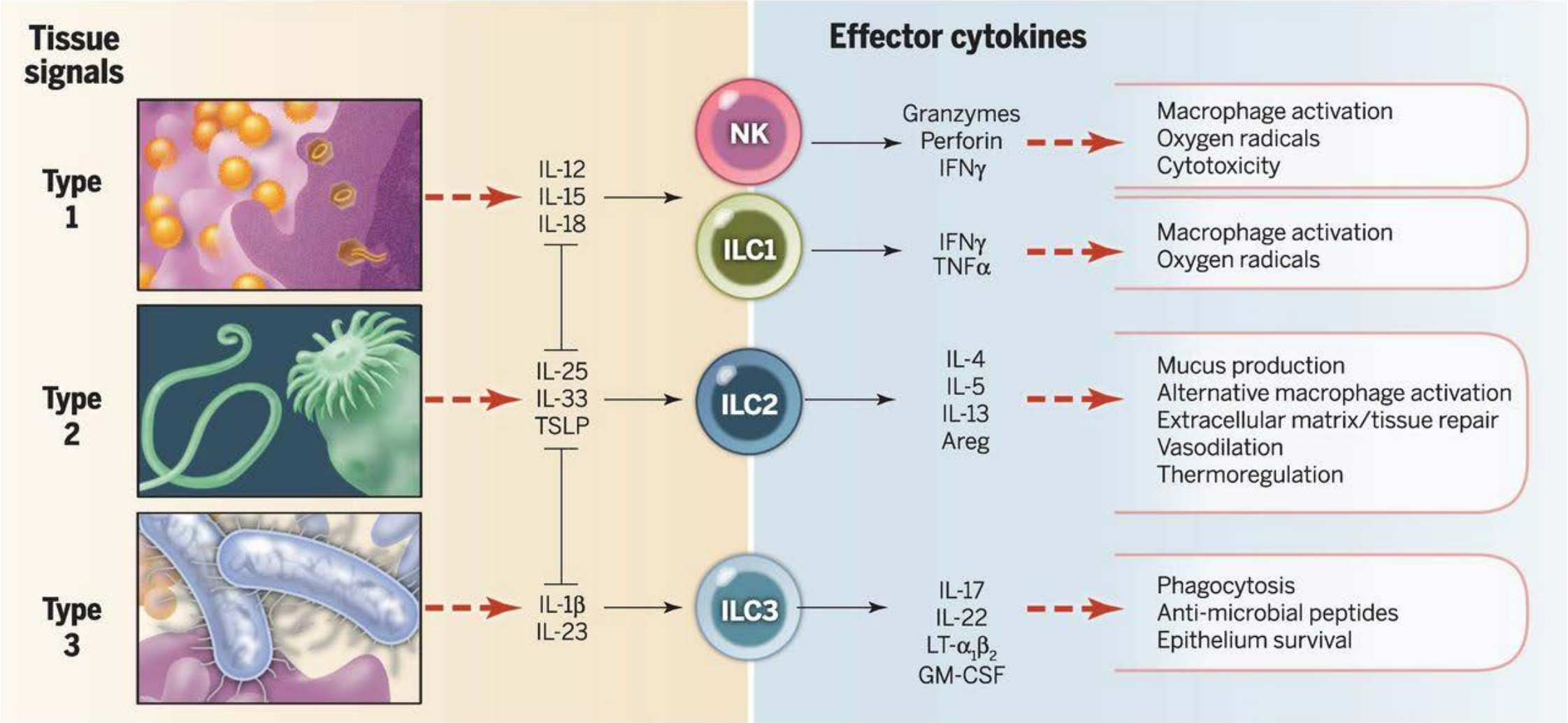
- Innate Lymphoid Cells: A new paradigm in immunology
- Group 2 innate lymphoid cells (ILC2s) in type 2 immunity and inflammation
- ILC2s in the development of non-atopic asthma and rhinitis (mouse model)
- Remaining questions and future research

What are Innate Lymphoid Cells (ILCs)?

- Newly discovered family of immune cells that do not express cell lineage (Lin) markers associated with T cells, B cells, dendritic cells, macrophages or granulocytes.
- ILCs mirror the phenotypes and functions of T cells.
- Unlike T cells, they do not express acquired antigen receptors or undergo clonal selection and expansion when stimulated (adaptive immunity).
- ILCs respond promptly to signals from infected or injured tissues and produce an array of secreted cytokines that direct the developing immune response to the insult (e.g., pathogen, allergen, toxin).

Eberl G et al. *Science*. 2015 May 22;348(6237):aaa6566.

ILCs are Effector Cells?



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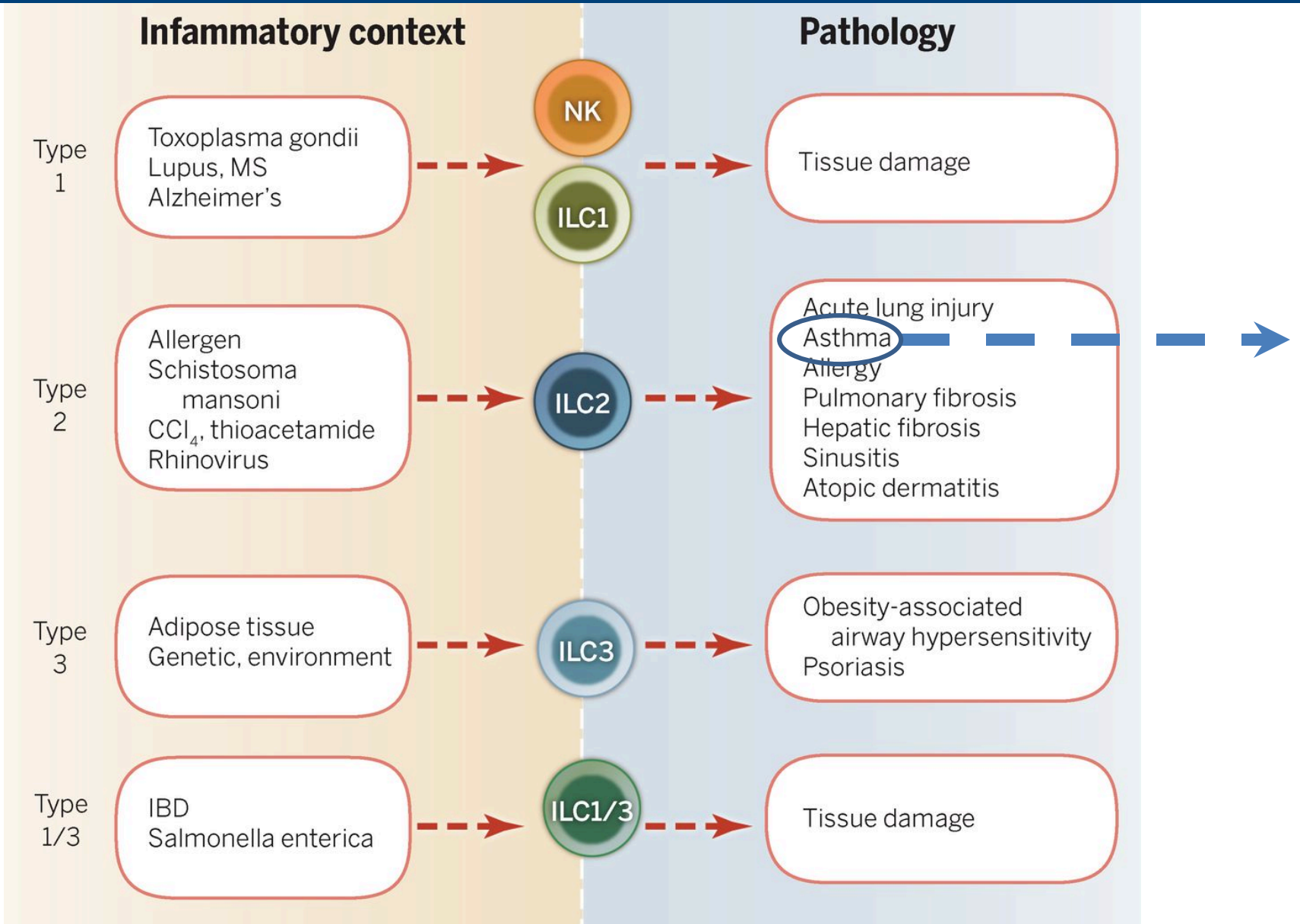
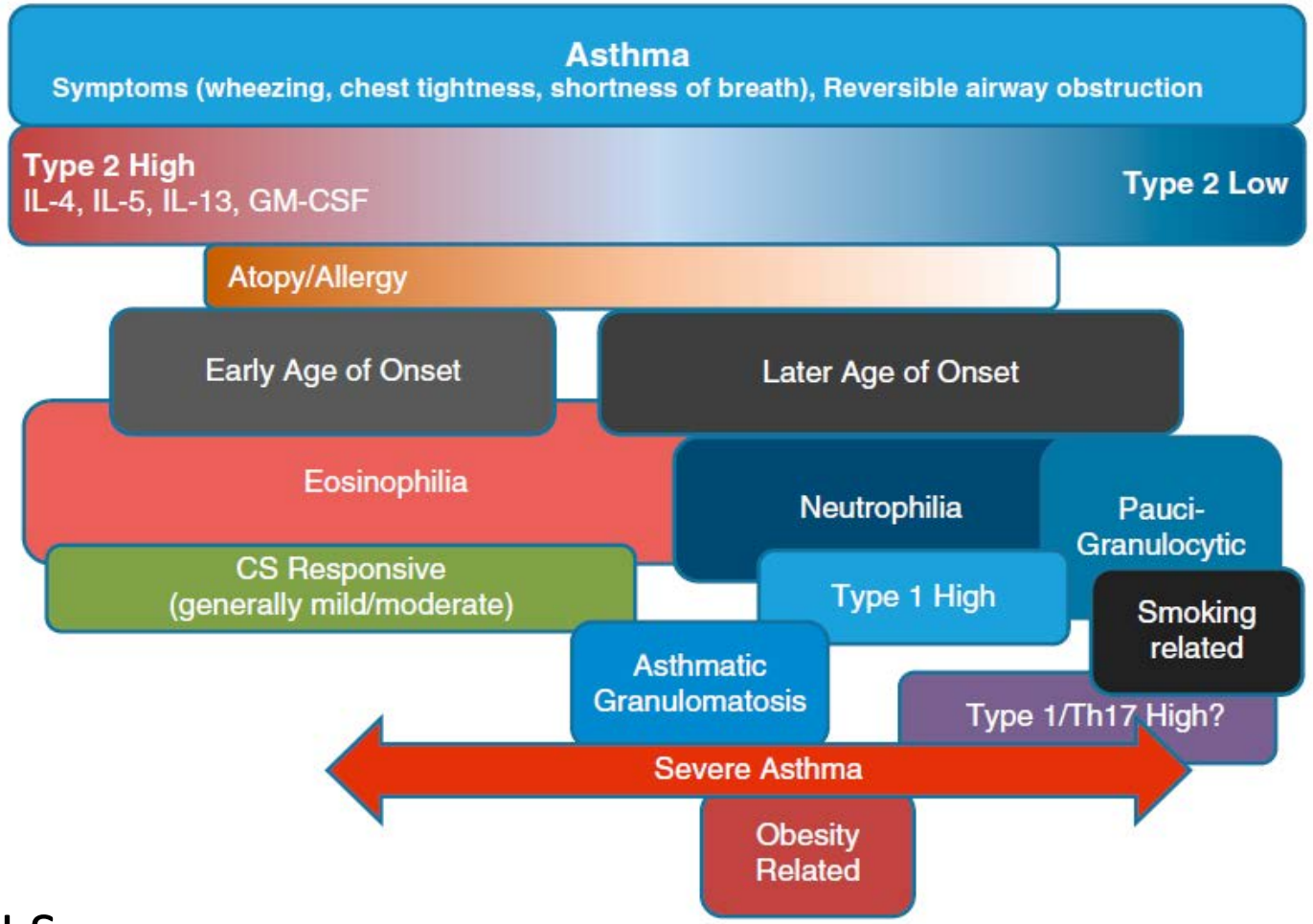
ILCs play a role in Pathology



Eberl G et al. *Science*. 2015 May 22;348(6237):aaa6566.

Evolving Concepts of Asthma: Many Phenotypes (not one disease)



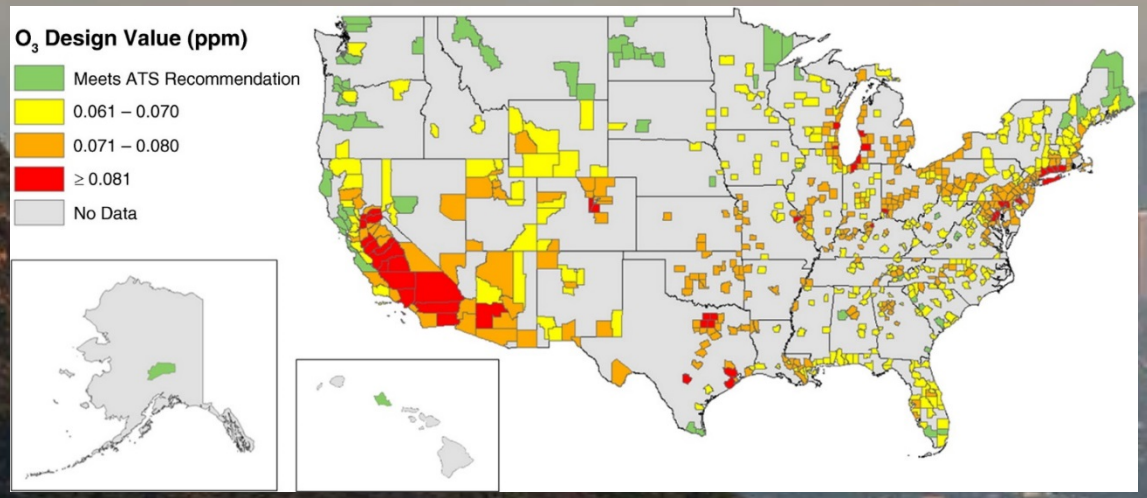
- Asthma is the most common chronic respiratory disease of children
- 6 million children have asthma in the U.S.
- 16.5 million asthmatic adults in the U.S.
- 300 million asthmatics worldwide

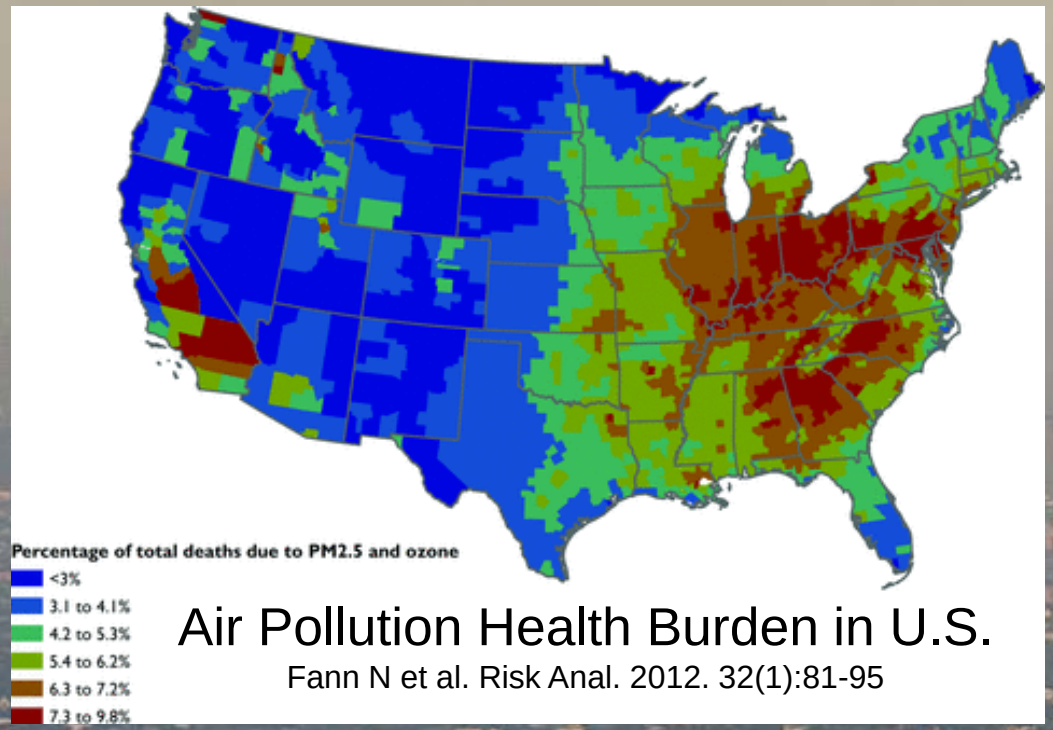
http://www.cdc.gov/asthma/most_recent_data.htm

Gauthier M, Ray A, Wenzel SE. Evolving Concepts of Asthma. Am J Respir Crit Care Med. 2015 Sep 15;192(6):660-8.

Air Pollution Associated with Exacerbation and New Onset of Asthma

Los Angeles, CA



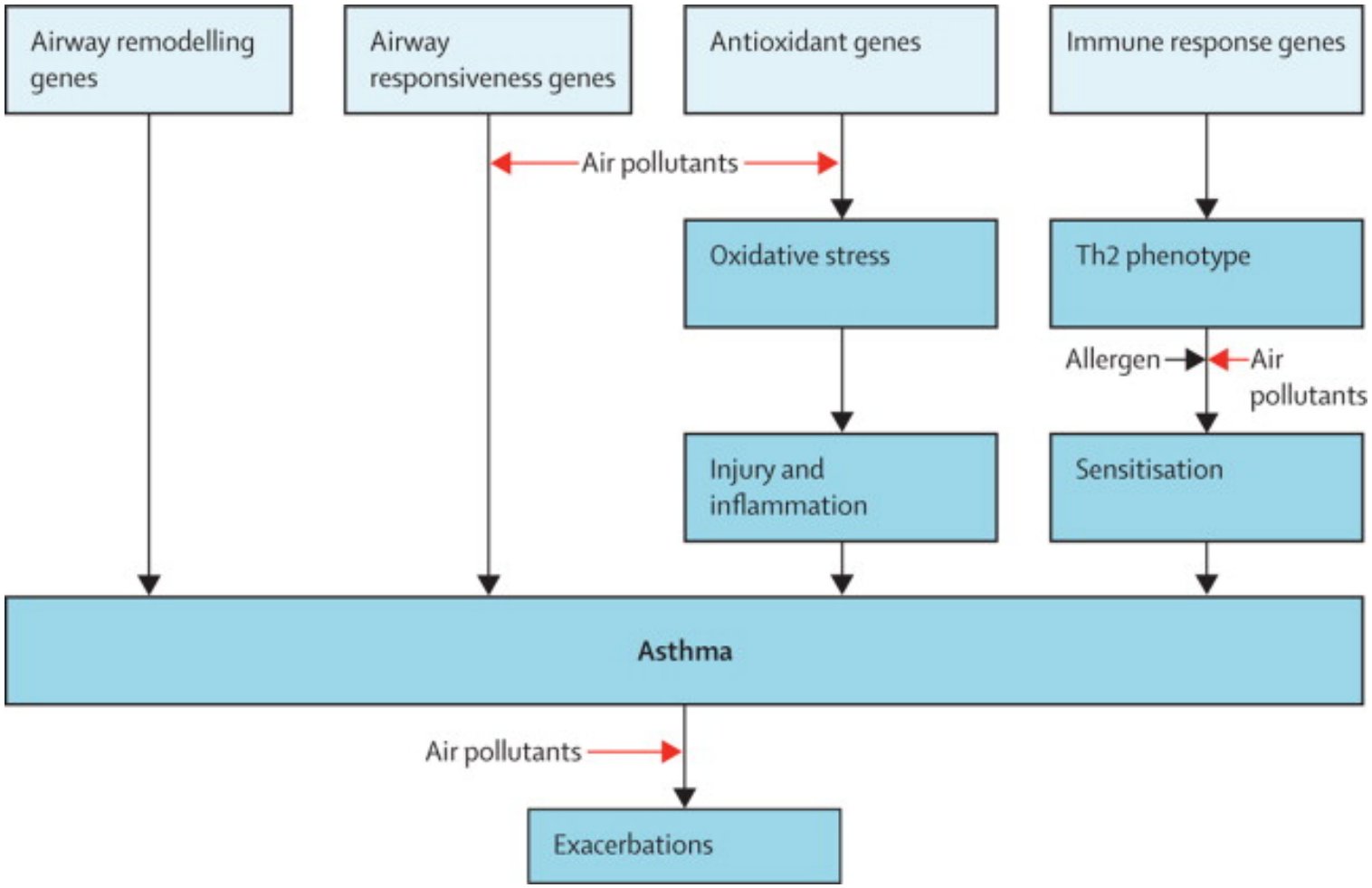


Air Pollution Health Burden in U.S.

Fann N et al. Risk Anal. 2012. 32(1):81-95

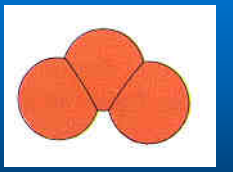
45% of the people in the United States (147.6 million) live in counties with unhealthy levels of either ozone or particle pollution.

Mechanistic Framework for Air Pollutant Effects on Asthma



Guarnieri M, Balmes JR. Outdoor air pollution and asthma. Lancet. 2014 May 3;383(9928):1581-92.

Ozone (O_3) and Health Effects



- One of the most reactive chemicals
- Oxidant gas in photochemical smog (secondary pollutant)
- 40-50% of the U.S. population live in communities where average ambient concentrations exceed the NAAQS
- Respiratory toxicant causing pulmonary function decrements, airway inflammation and remodeling
- Causes various extrapulmonary effects (e.g., rhinitis)
- Contributes to both morbidity and mortality

Ozone and Asthma Research

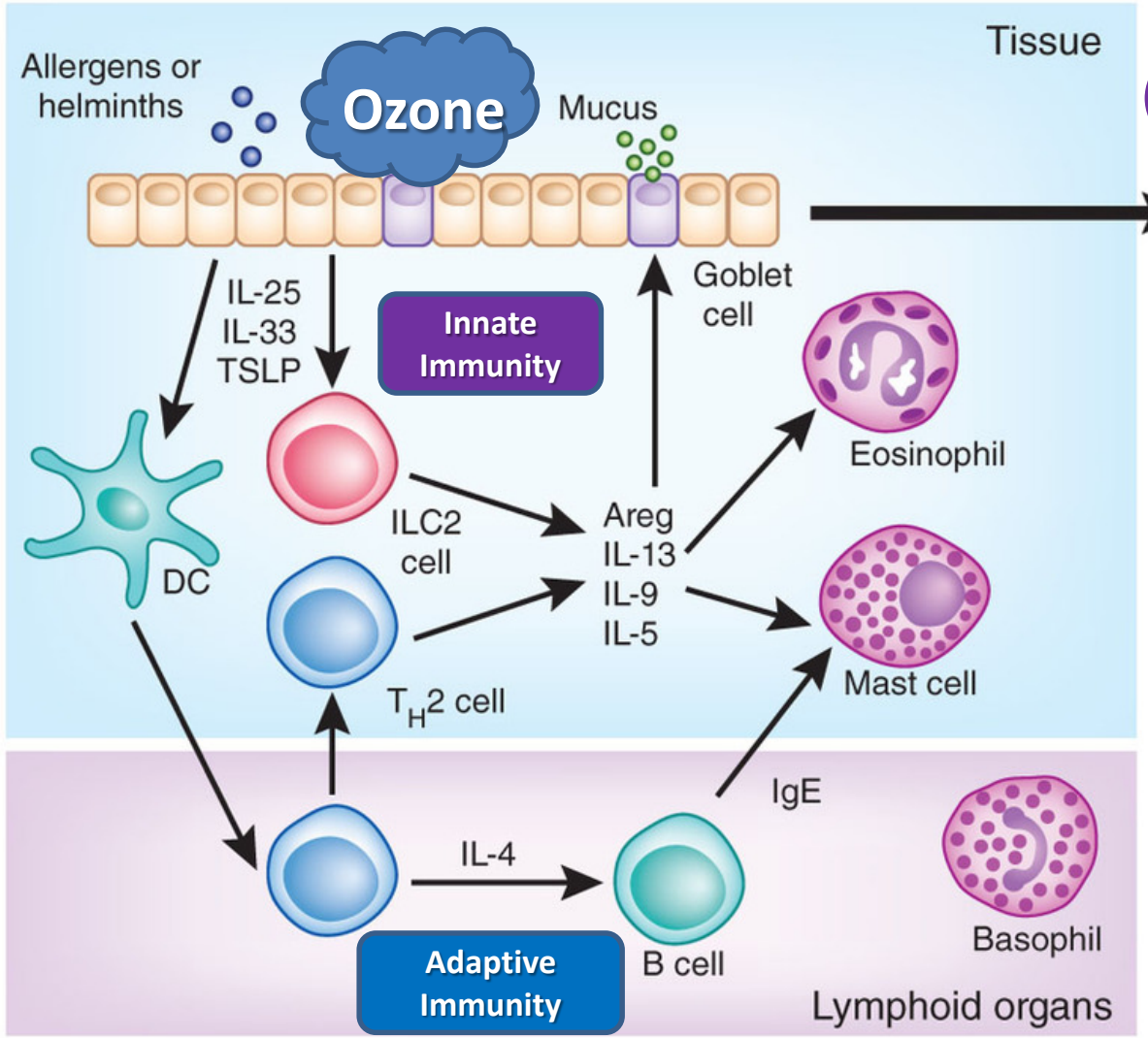
- Epidemiological associations have been found between elevated ambient concentrations of ozone and

  1) Onset of asthma in non-atopic Latino children (Nishimura et al., 2016)

  2) Eosinophilic airway inflammation in children without atopy (e.g., Frischer et al., 1993, 2001)

- In mice, repeated ozone exposures cause eosinophilic rhinitis, nasal epithelial remodeling, and other airway responses characteristic of type 2 immunity/inflammation.*

***Ong CB et al. Am J Respir Cell Mol Biol. 2016 Mar; 54 (3): 331-40. Kumagai K, *et al.* Am J Respir Cell Mol Biol. 2016 Jun; 54 (6): 782-91.**



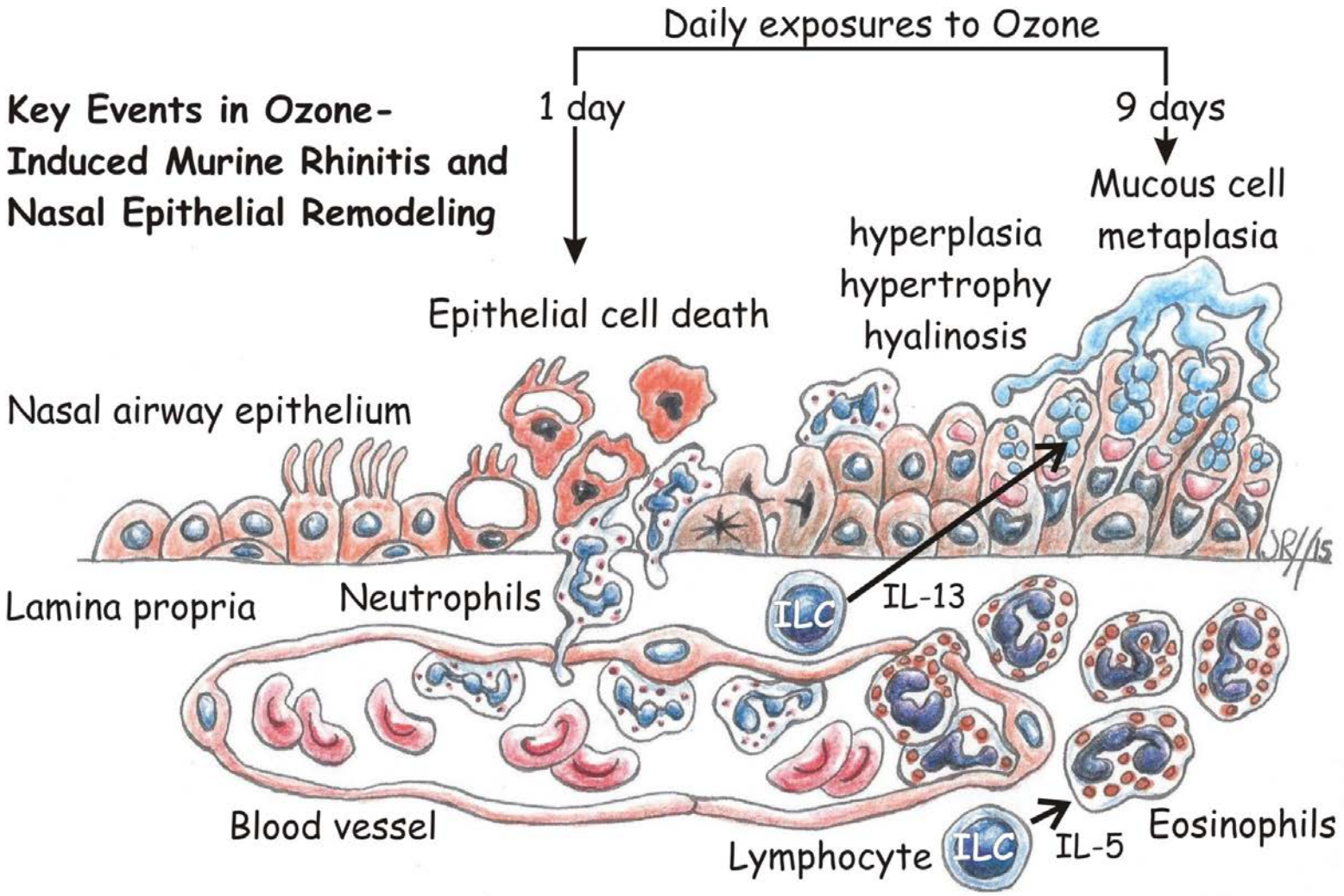
Non-Atopic Asthma

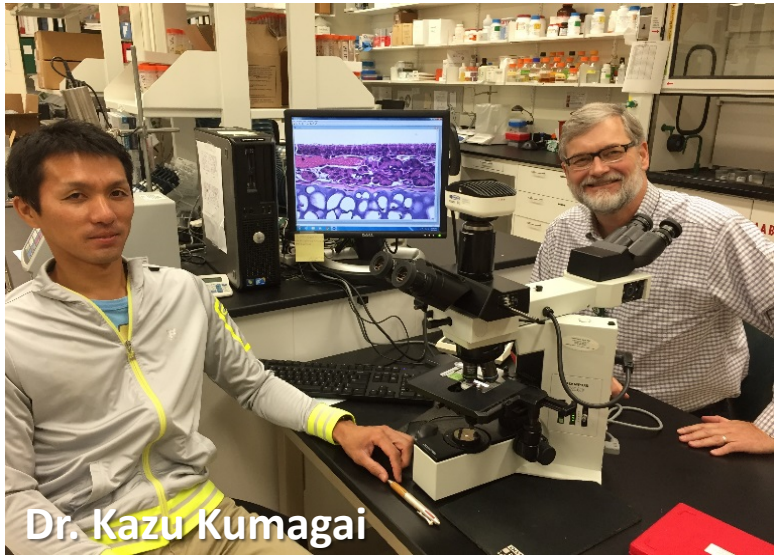
Influx of eosinophils
Mucous cell metaplasia
Epithelial hyperplasia
Ym1/2 proteins
Airway Hyper-responsiveness

Initiation and propagation of type 2 immune responses

Modified from Licona-Limón et al., Nat Immunol 14: 536-542, 2013

New Paradigm: Ozone-induced Non-Atopic Rhinitis and Asthma is ILC2-Dependent

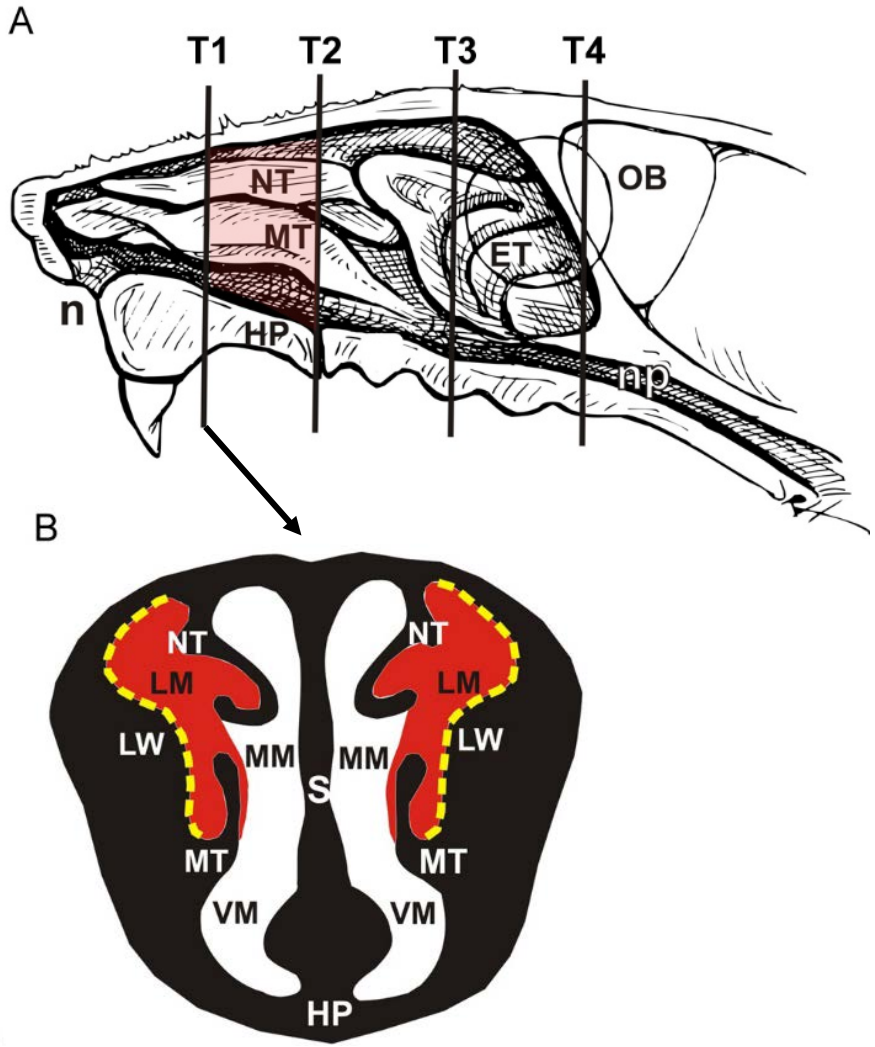




Ong CB et al. Am J Respir Cell Mol Biol. 2016 Mar; 54 (3): 331-40. Kumagai K, *et al.* Am J Respir Cell Mol Biol. 2016 Jun; 54 (6): 782-91.

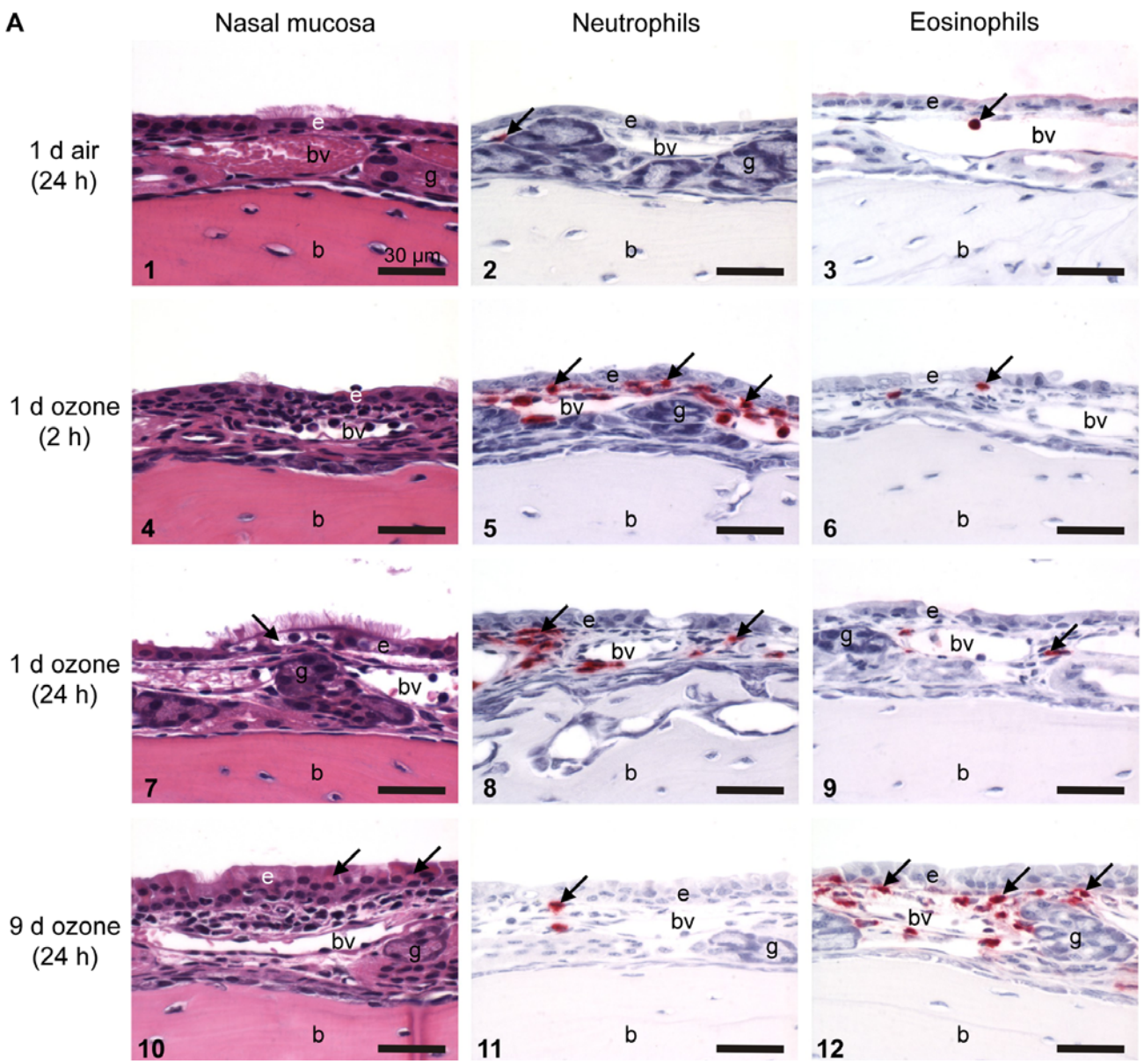
Aim 1: To determine the onset of ozone-induced eosinophilic rhinitis and nasal type 2 immunity

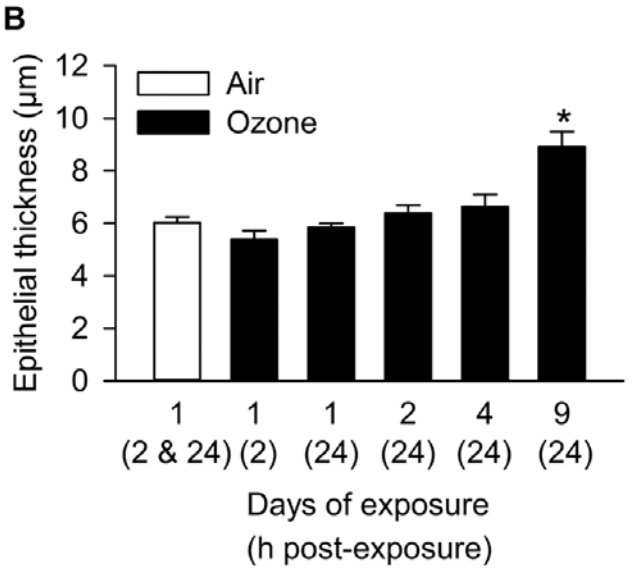
- C57BL/6 male mice
- 0 or 0.5 ppm ozone (4h/day) for 1, 2, 4 or 9 weekdays
- Nasal histopathology
- Immunohistochemistry and morphometric analysis
- qRT-PCR for relative mRNA expression of selected inflammatory cytokines and airway epithelial proteins

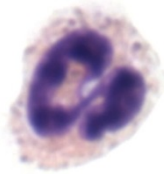


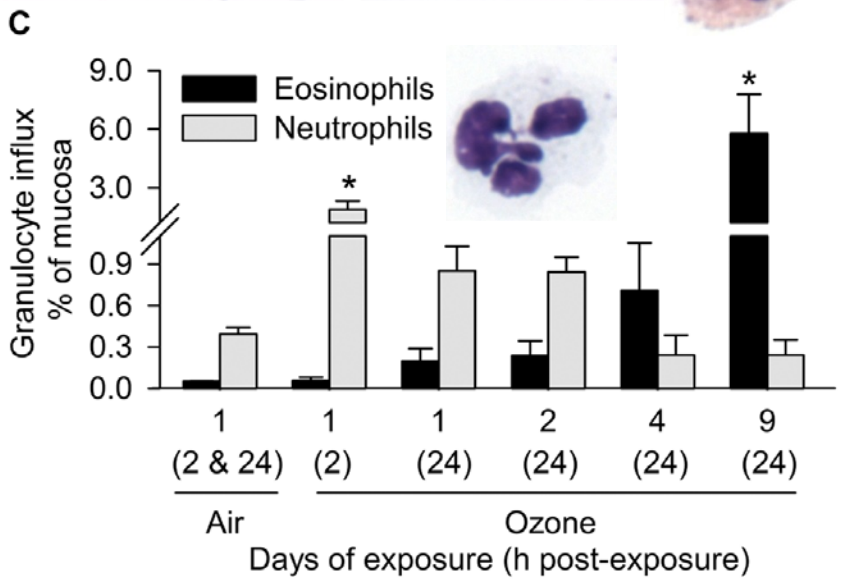
Ong CB et al. Am J Respir Cell Mol Biol. 2016 Mar; 54 (3): 331-40.

Nasal Epithelial Thickness and Granulocytes with Increasing Days of Exposure

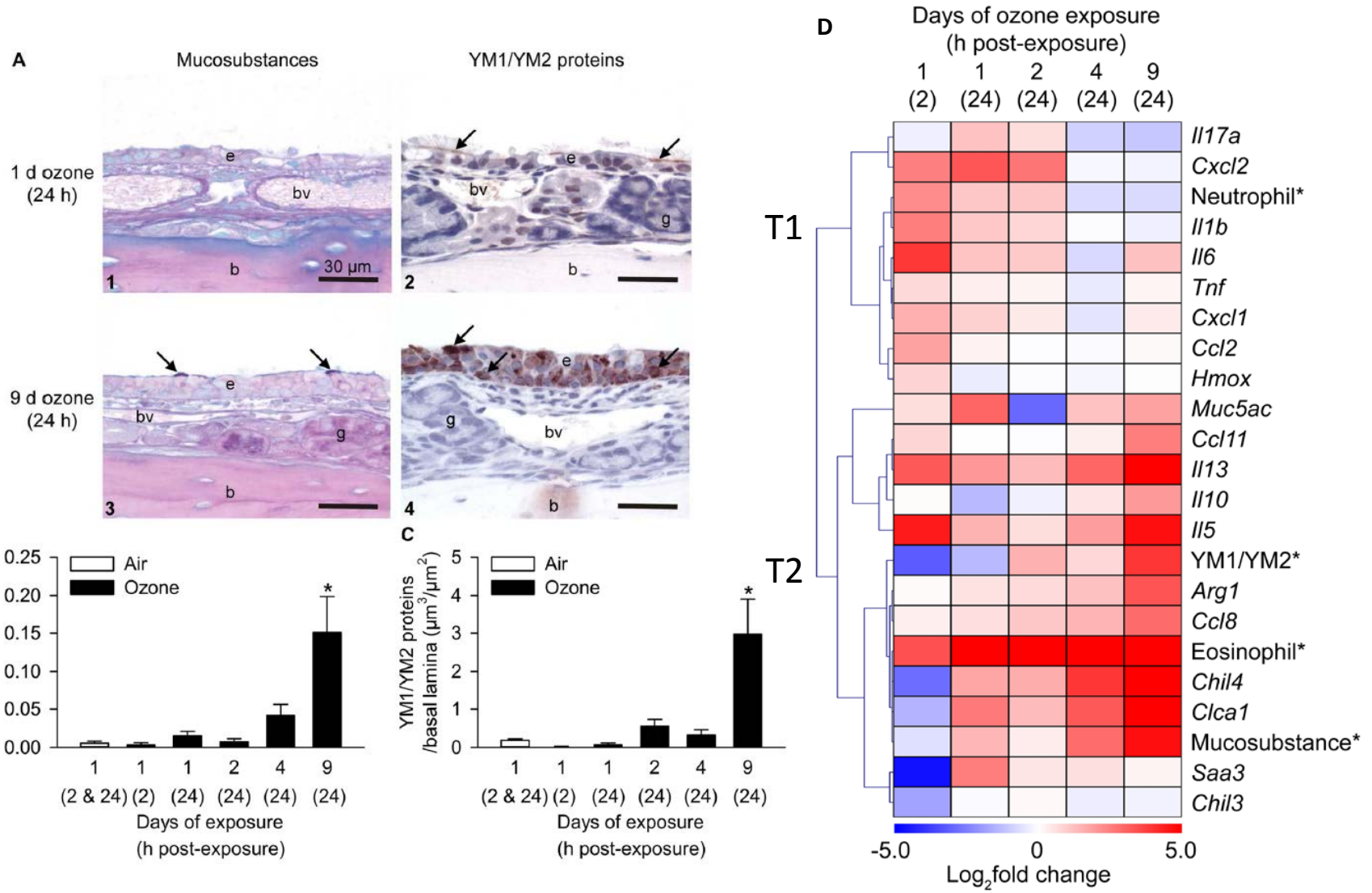








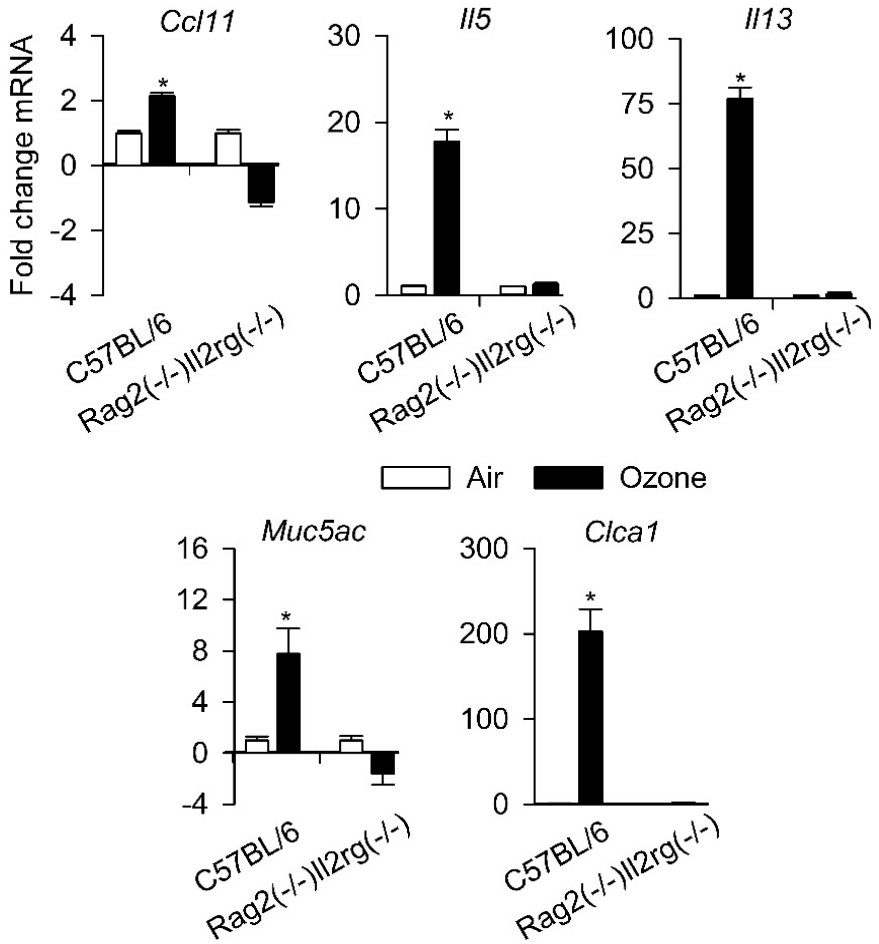
Nasal Epithelial Protein and Gene Expression with Increasing Days of Exposure

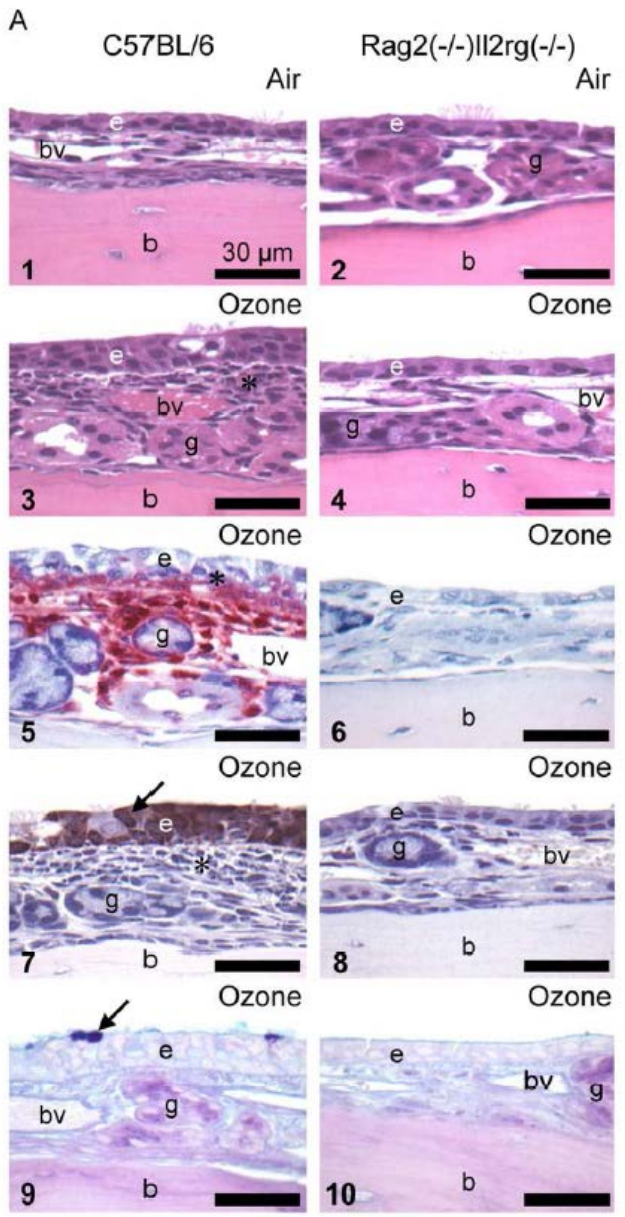


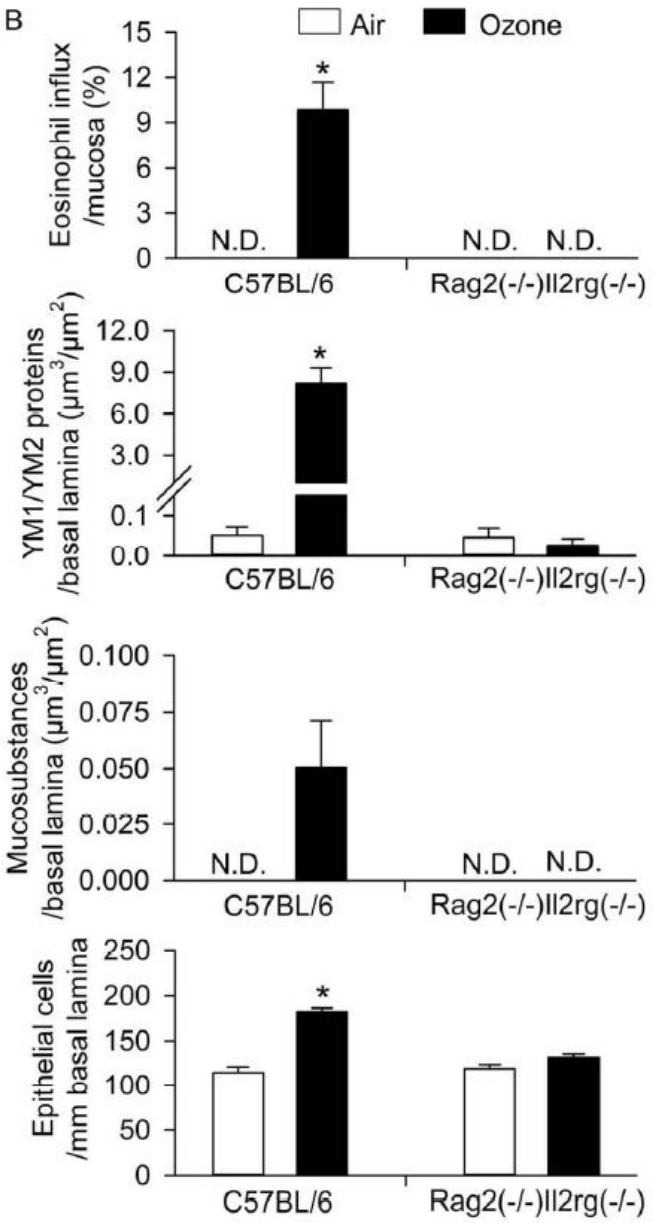
Ong CB et al. Am J Respir Cell Mol Biol. 2016 Mar; 54 (3): 331-40.

Aim 2: To determine the role of lymphoid cells in ozone-induced eosinophilic rhinitis and nasal type 2 immunity

- Lymphoid cell-deficient Rag2(-/-)IL2rg (-/-) and Lymphoid cell-sufficient C57BL/6 mice
- 0 or 0.5 ppm ozone (4h/day) for 9 days

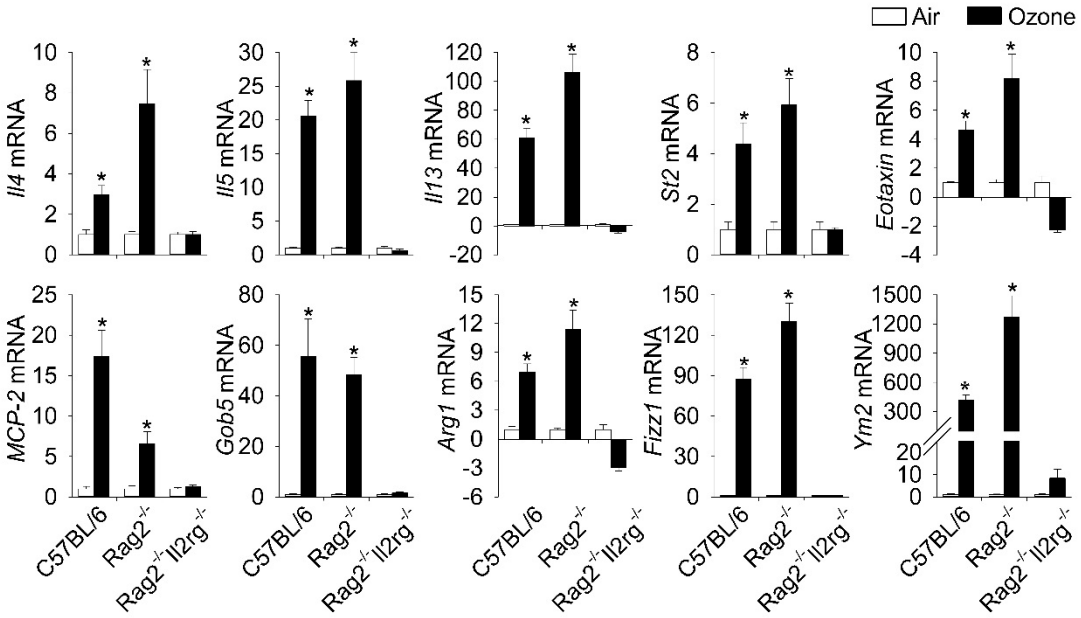


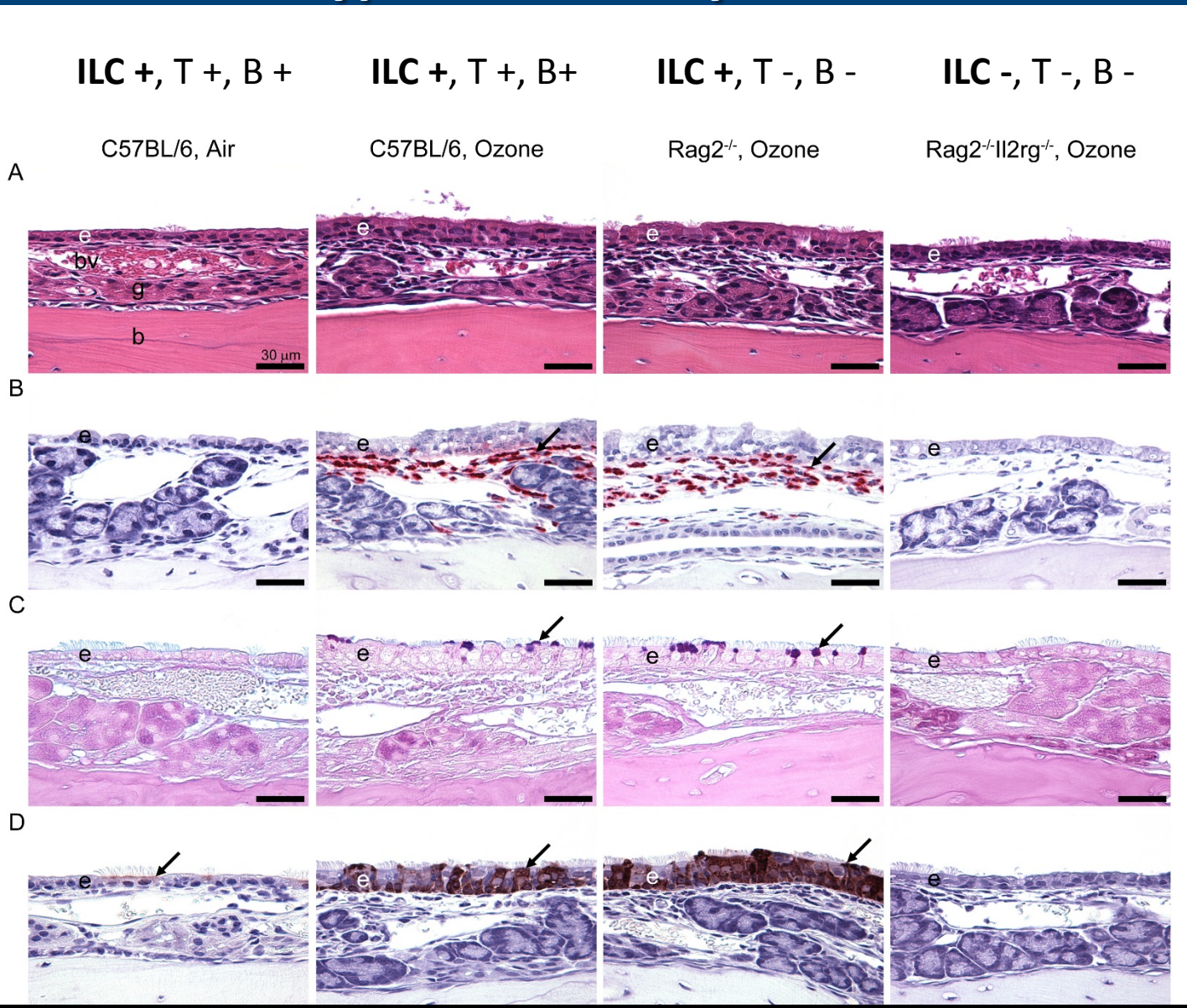




Aim 3: Determine the role of ILCs in ozone-induced eosinophilic rhinitis and nasal type 2 immunity

- Lymphoid cell-deficient Rag2(-/-) IL2rg(-/-), lymphoid cell-sufficient C57BL/6 mice, ILC-sufficient and T & B cell-deficient Rag2(-/-) mice
- 0 or 0.8 ppm ozone (4h/day) for 9 days



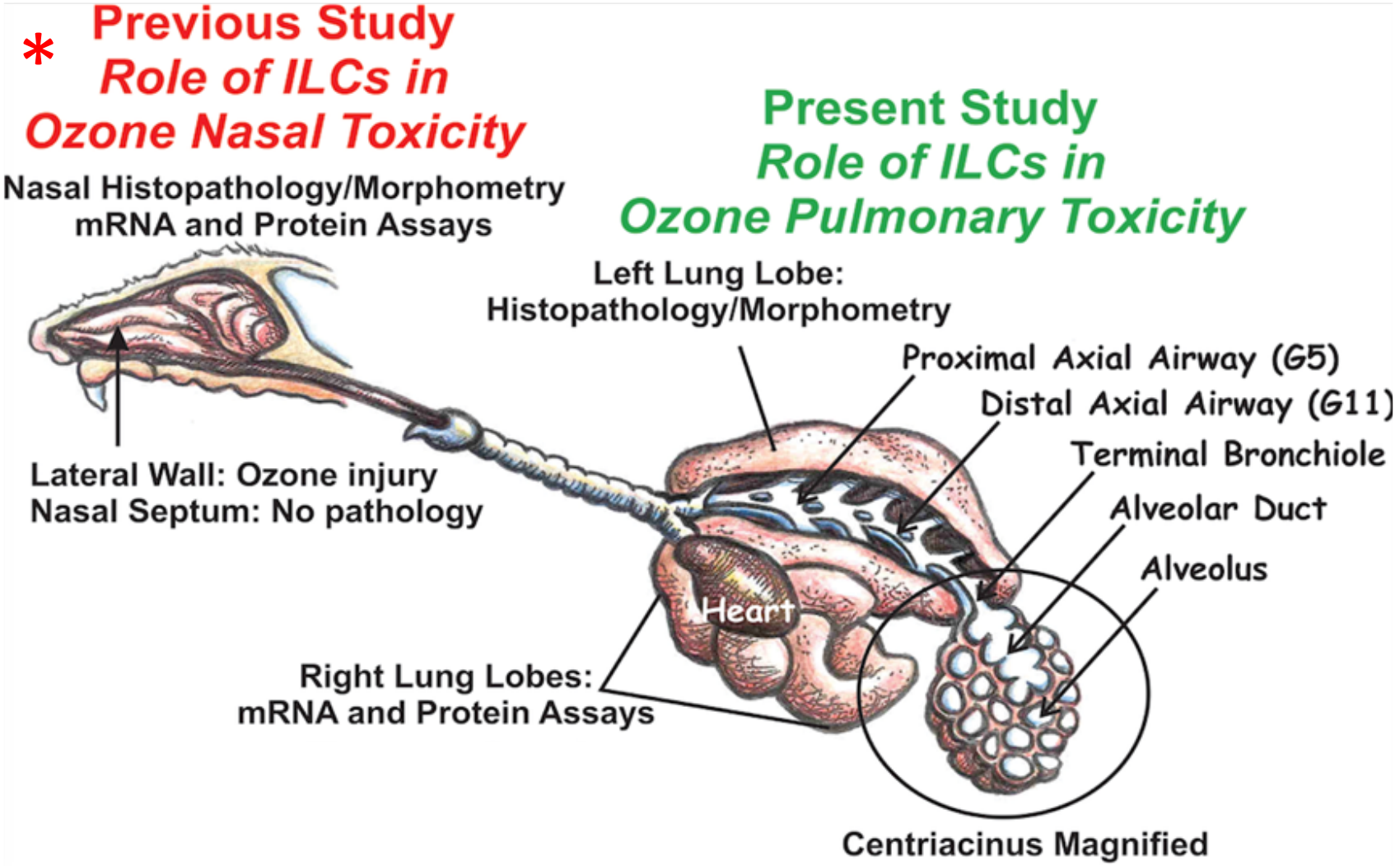


Kumagai K, *et al.* Am J Respir Cell Mol Biol. 2016 Jun; 54 (6): 782-91.

Summary: Nasal Study

Mouse Strain	T & B cells	ILCs	O3-induced lesions
C57BL/6	+	+	+
Rag2(-/-)	-	+	+
Rag2(-/-)Il2rg(-/-)	-	-	-

Repeated exposures to ozone elicit innate-type allergy in the nose of mice, that is likely to be dependent on type 2 cytokine-producing ILCs. This suggests a new biologic paradigm that underlies the epidemiologic association of ambient ozone exposure and eosinophilic rhinitis in non-atopic children.



*Kumagai K, *et al.* Am J Respir Cell Mol Biol. 2016 Jun; 54 (6): 782-91.

Hypothesis and Specific Aims

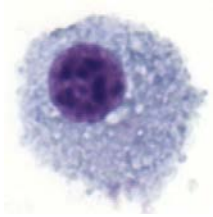
- **Hypothesis**: Mice repeatedly exposed to ozone will develop **pulmonary lesions** (e.g., eosinophilic inflammation, mucous cell metaplasia) that are dependent on ILCs.
- **Aim 1**: To determine the role of ILCs in **acute lung injury** (**single ozone exposure**; neutrophilic inflammation, airway epithelial cell death and reparative DNA synthesis).
- **Aim 2:** To determine the role of ILCs in **subacute lung injury** (**repeated ozone exposures**; eosinophilic inflammation and mucous cell metaplasia of airway epithelium).

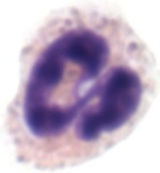
Methods: Animals and Exposures

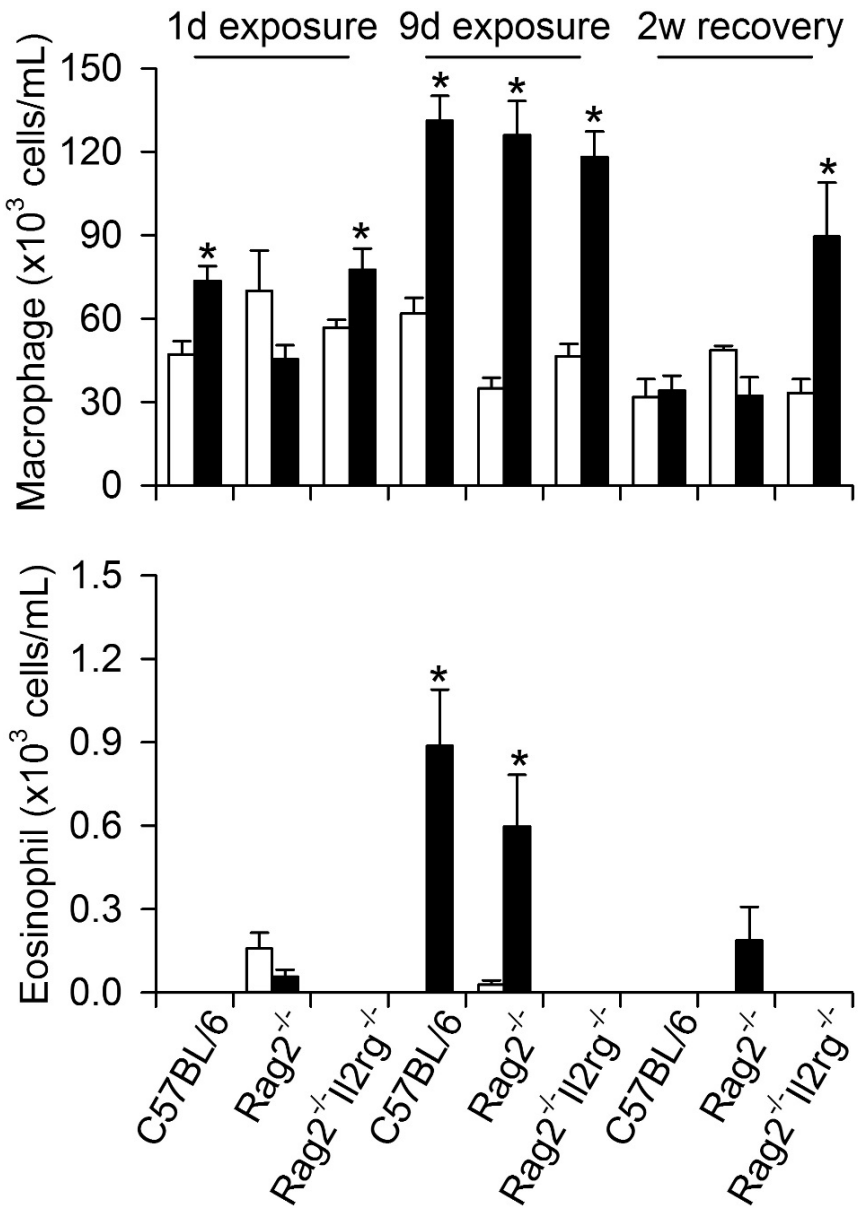
Mouse Strain 8wks old, 6mice/group	T and B Lymphoid Cells	Innate Lymphoid Cells
C57BL/6	+	+
Rag2-/-	–	+
Rag2-/-IL2rg-/-	–	–

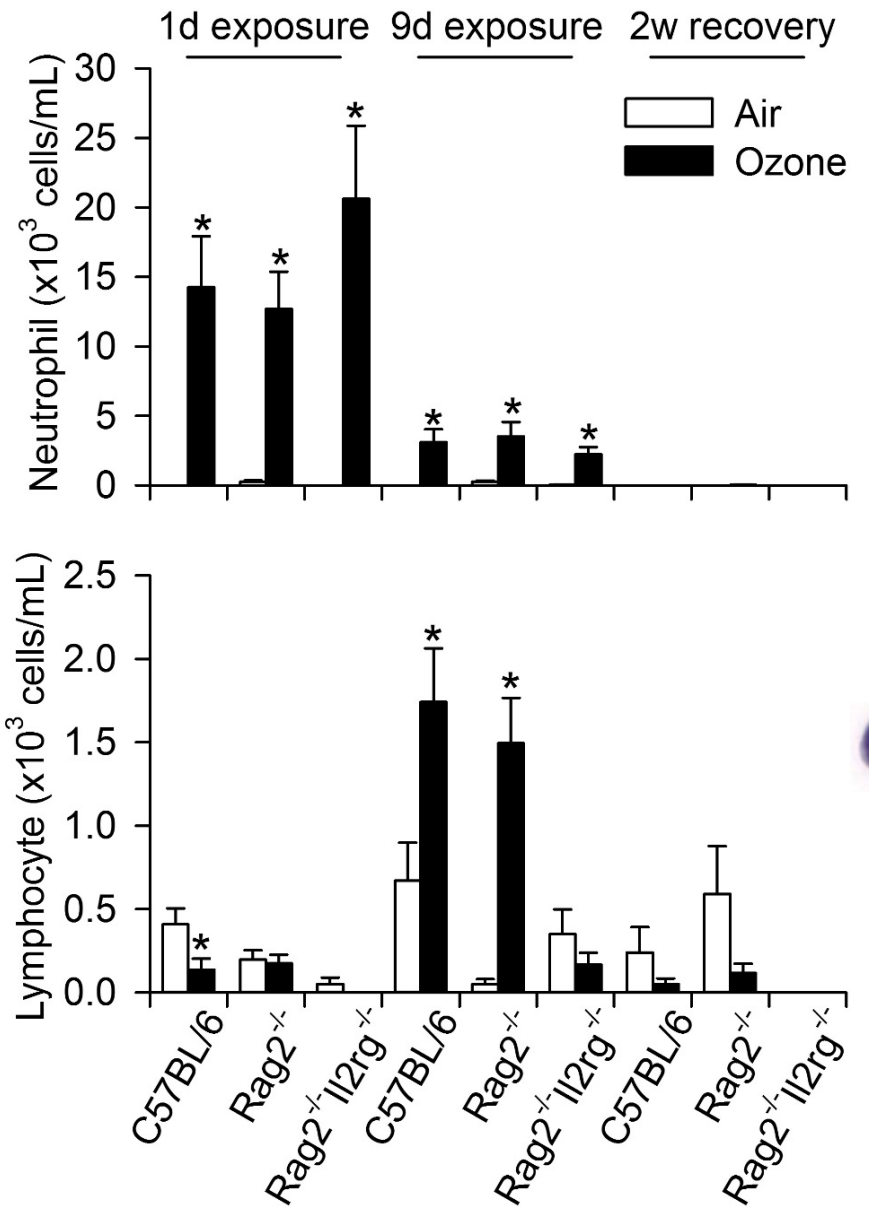
Ozone Chamber Concentration	Days of Exposure (4h/day)	
0 ppm (Filtered Air)	1 day	9 days
0.8 ppm	1 day	9 days

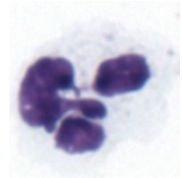
Results: Inflammatory Cells in BALF

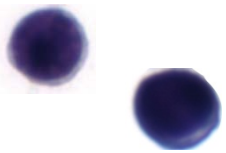






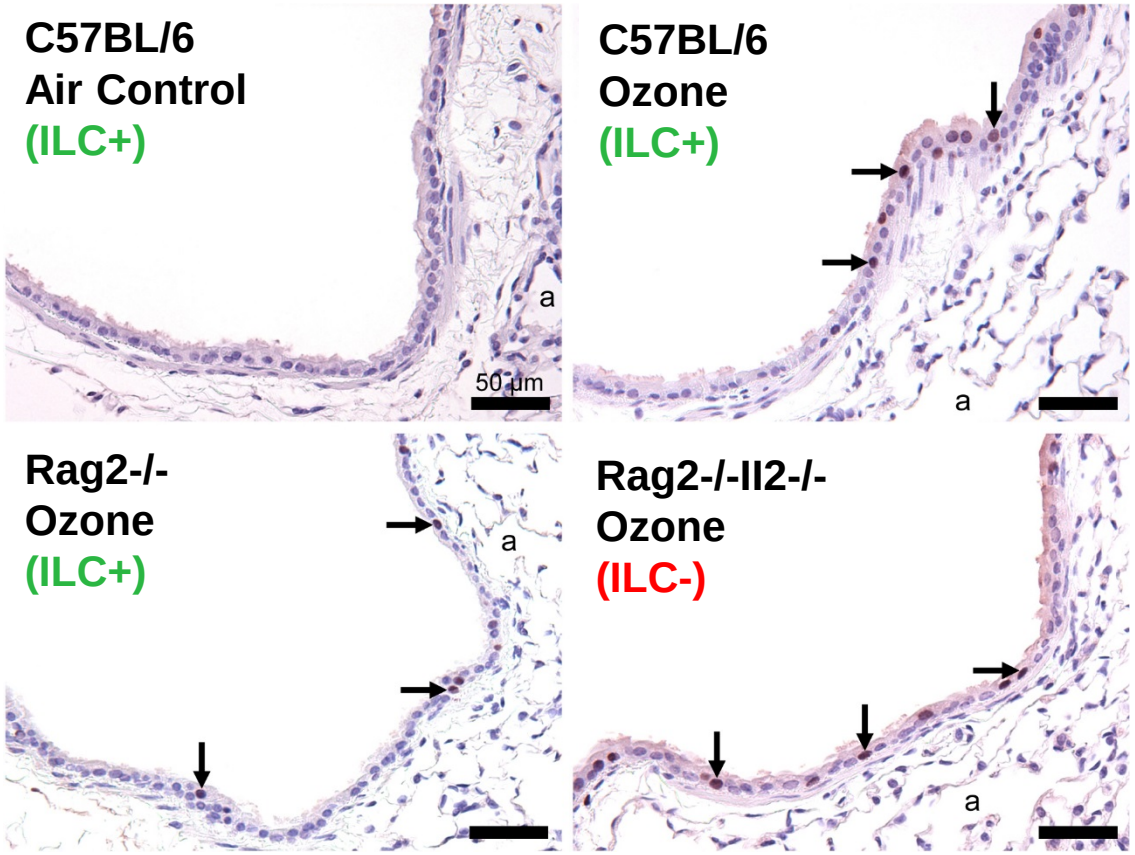


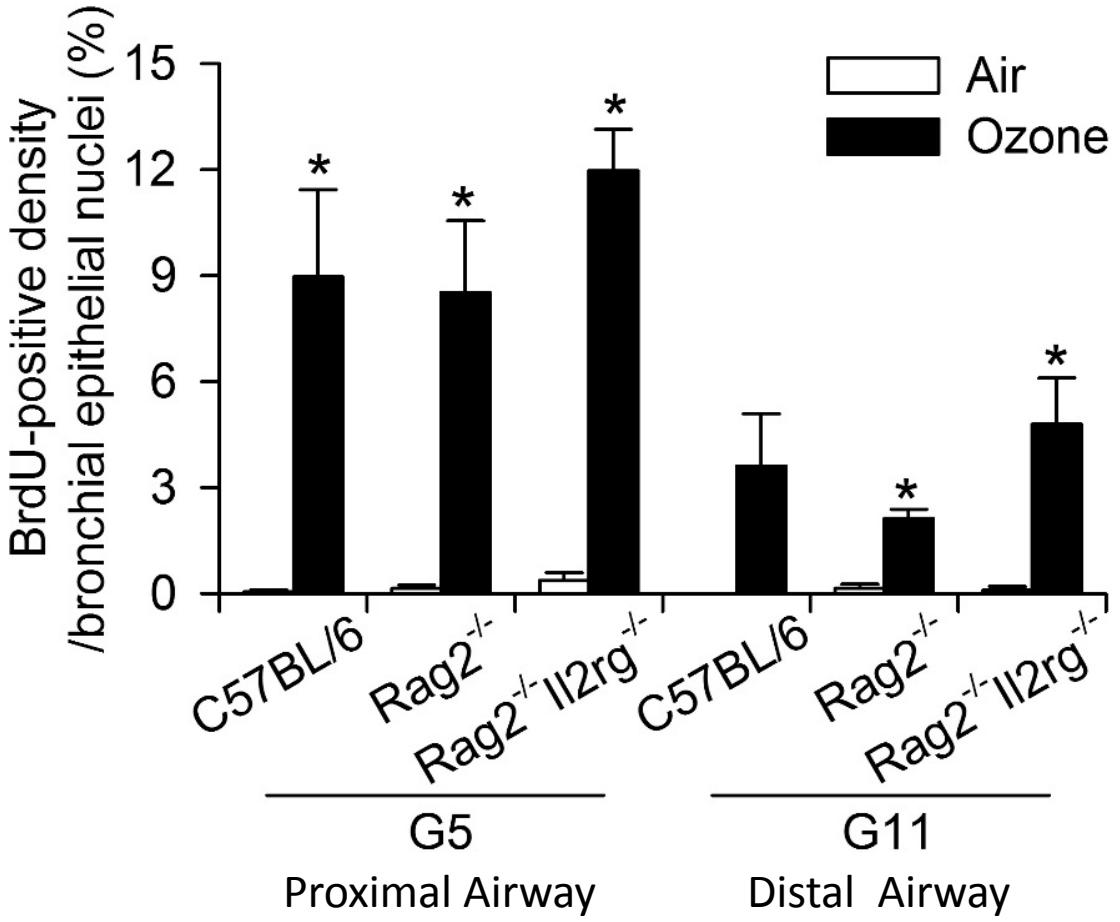




Ozone-induced DNA Synthesis in Bronchiolar Epithelium (1-day, 4-h, exposure)

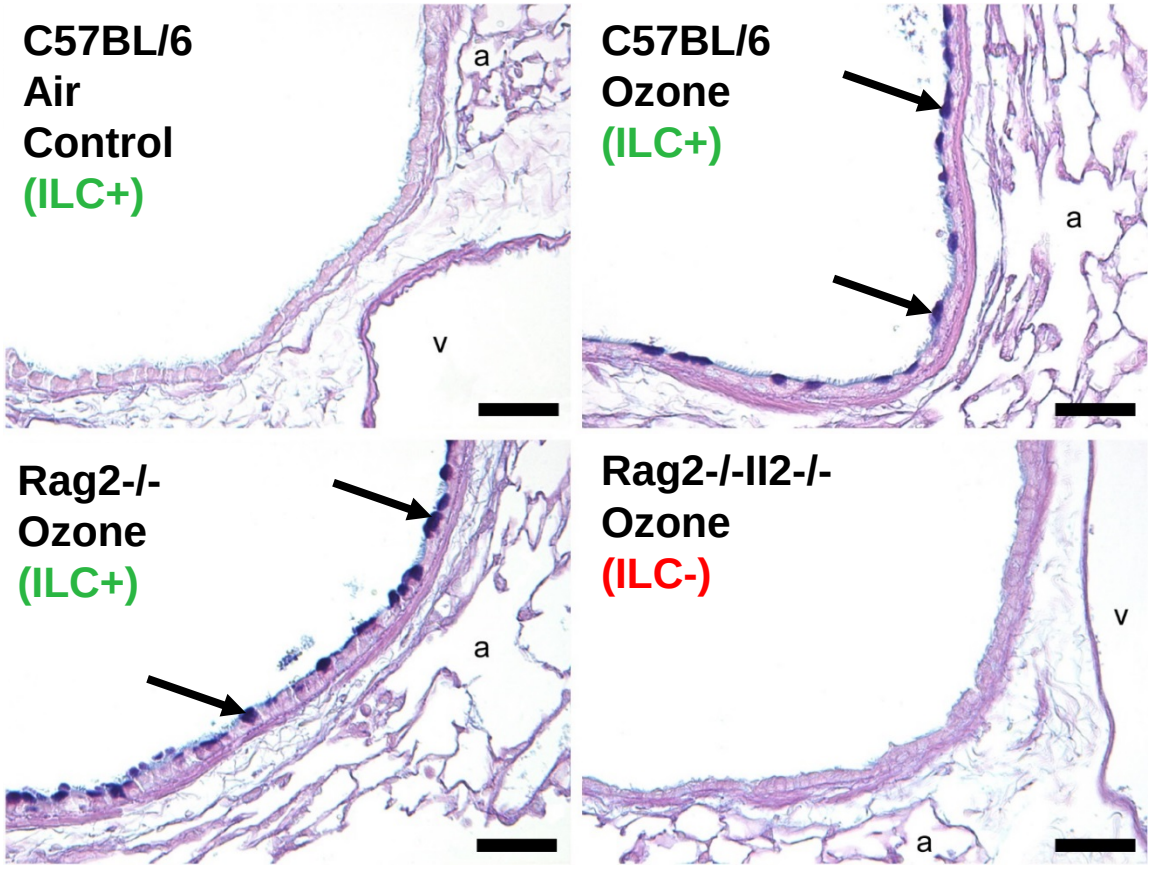
BrdU-labelled Epithelial Nuclei in Proximal Airways

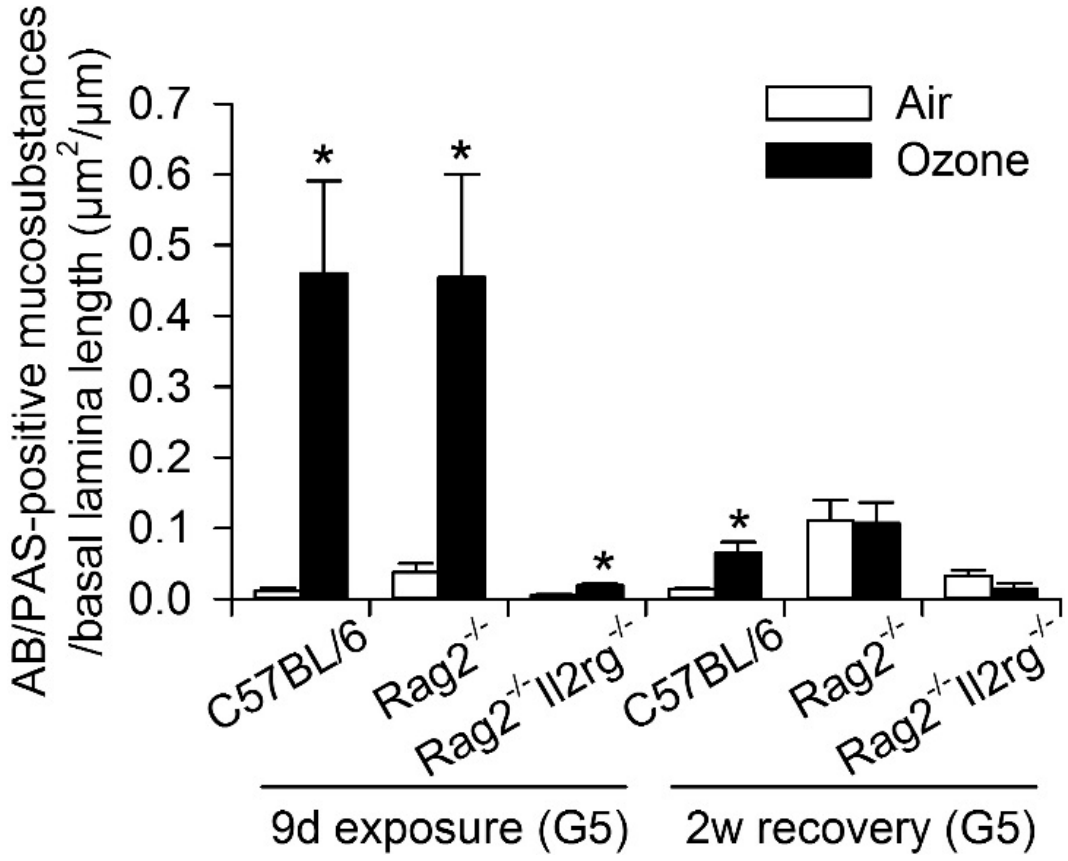




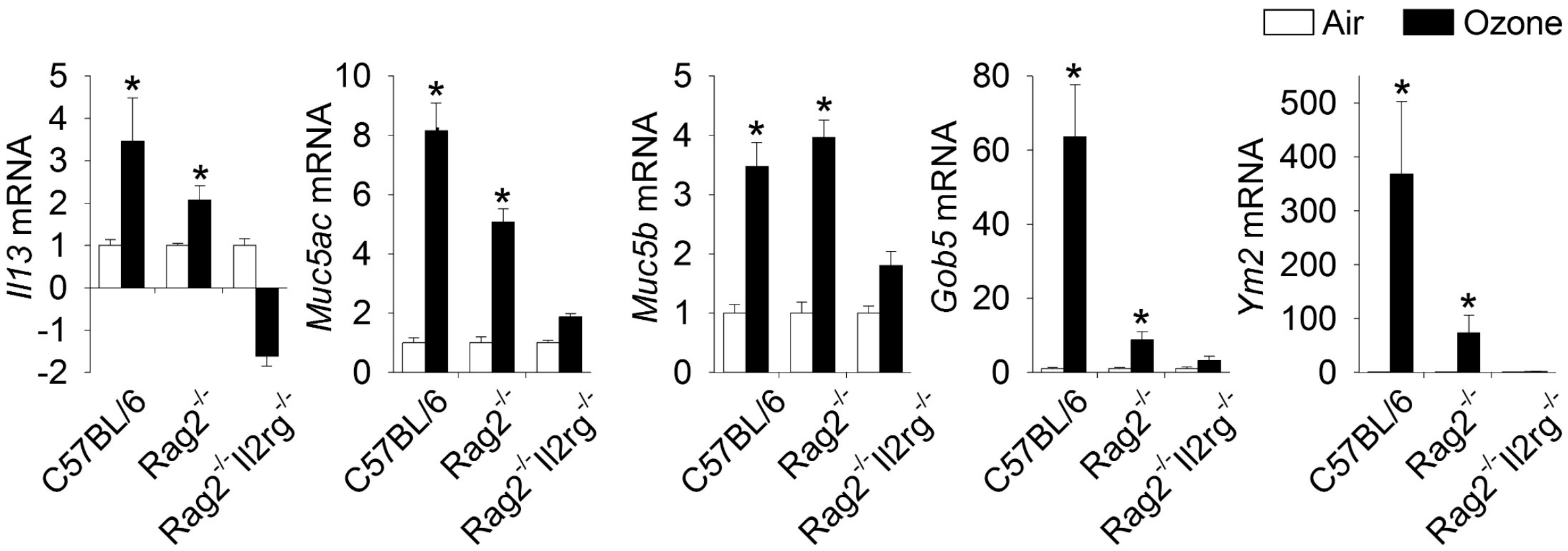
Ozone-induced Mucous Cell Metaplasia in Bronchiolar Epithelium (9-day exposure)

Alcian Blue/Periodic Acid Schiff-stained Mucosubstances in Proximal Airway Epithelium

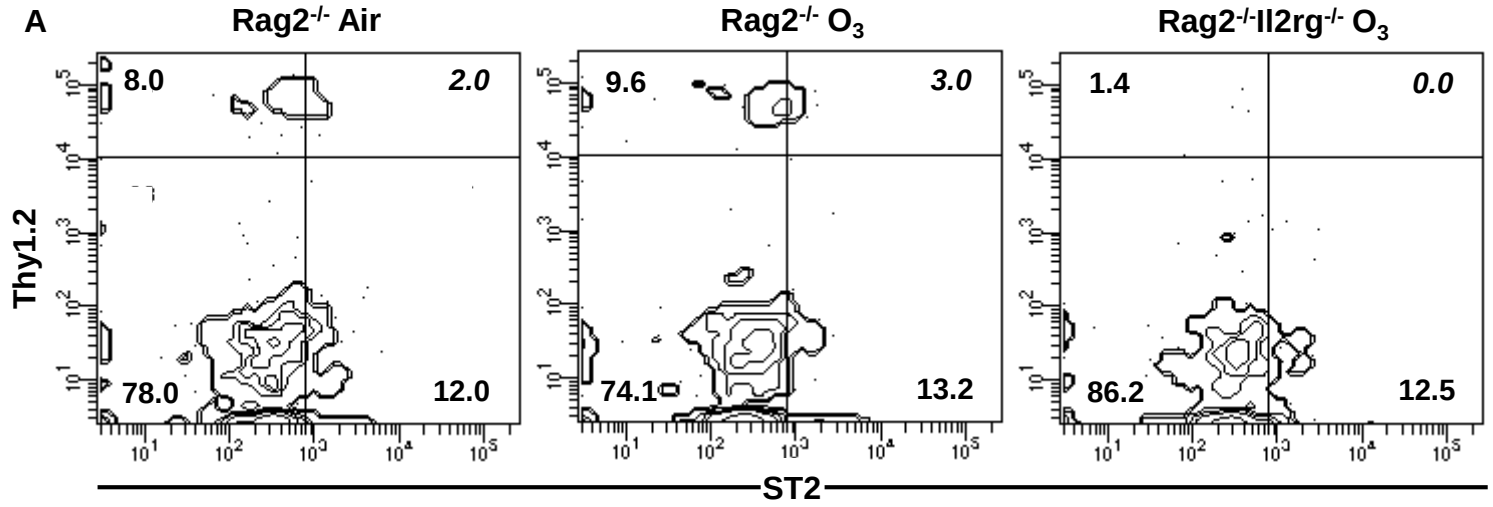


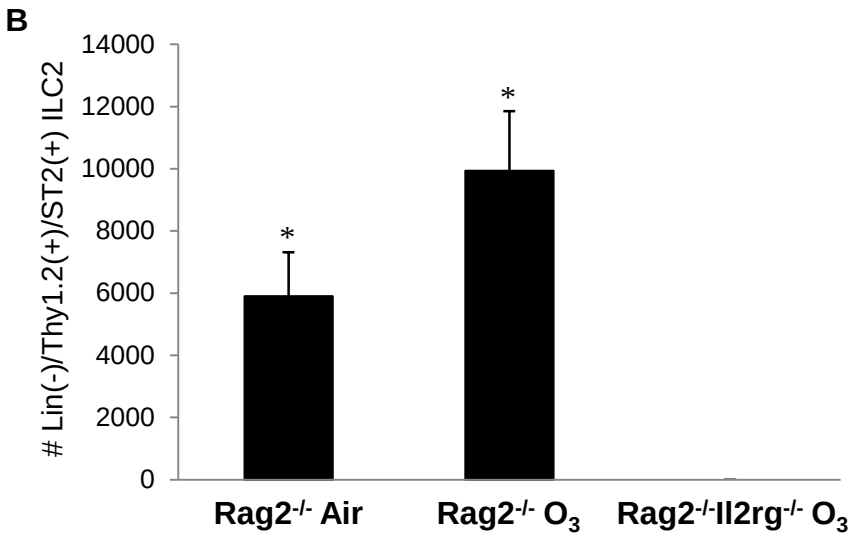


Mucus and Type 2 Immune-Related Gene Expression in the Lungs after 9 Days of Ozone Exposure



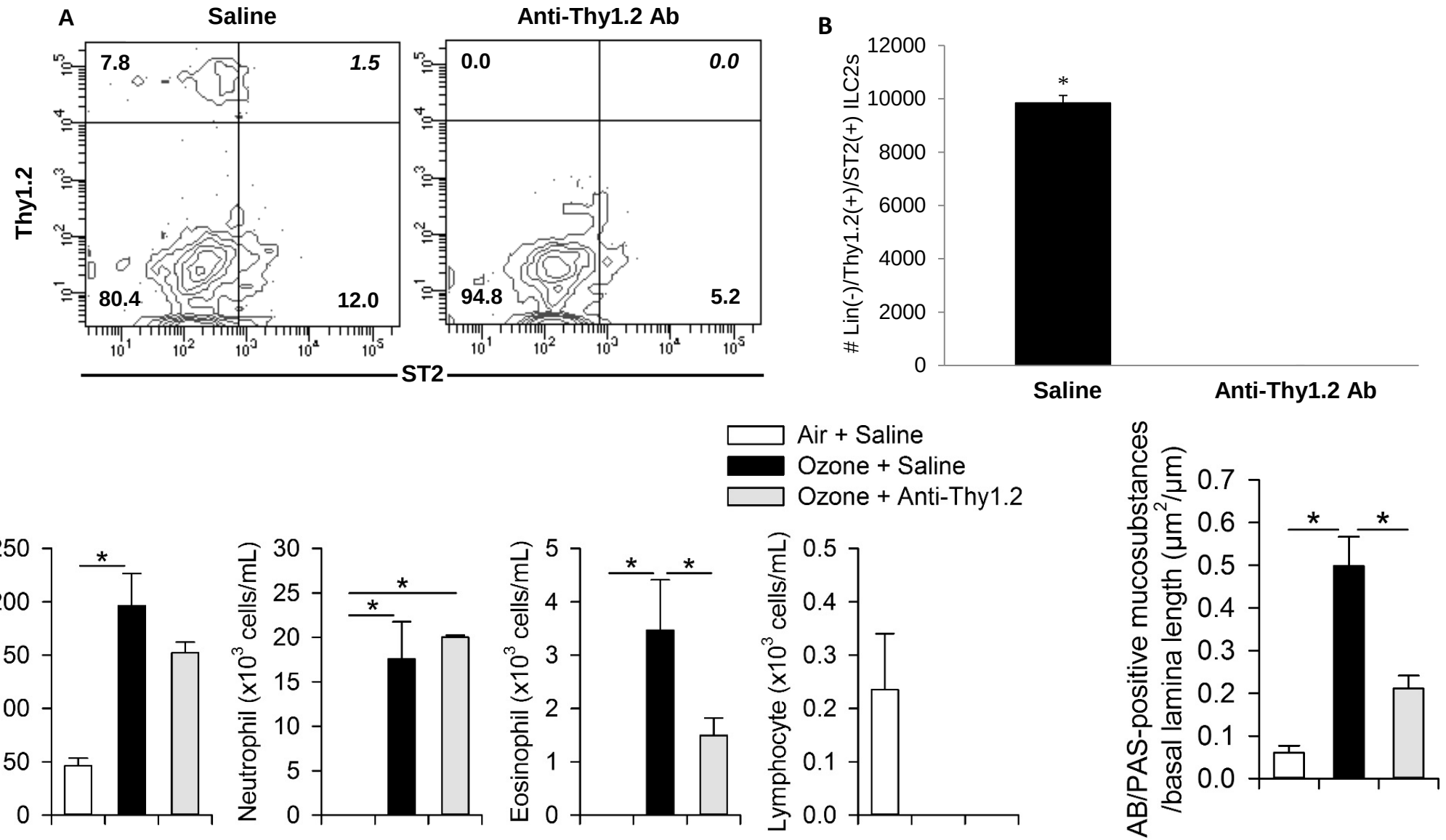
Flow Cytometry: ILC2s in the Lungs of Rag2-/- and Rag2-/-IL2rg-/- Mice exposed to Ozone





Exposure to ozone for 9 days did not have a statistically significant impact on the number of ILC2s in the lungs of mice.

Anti-Thy 1.2 Antibody-Treated Rag2-/- Mice exposed to Ozone



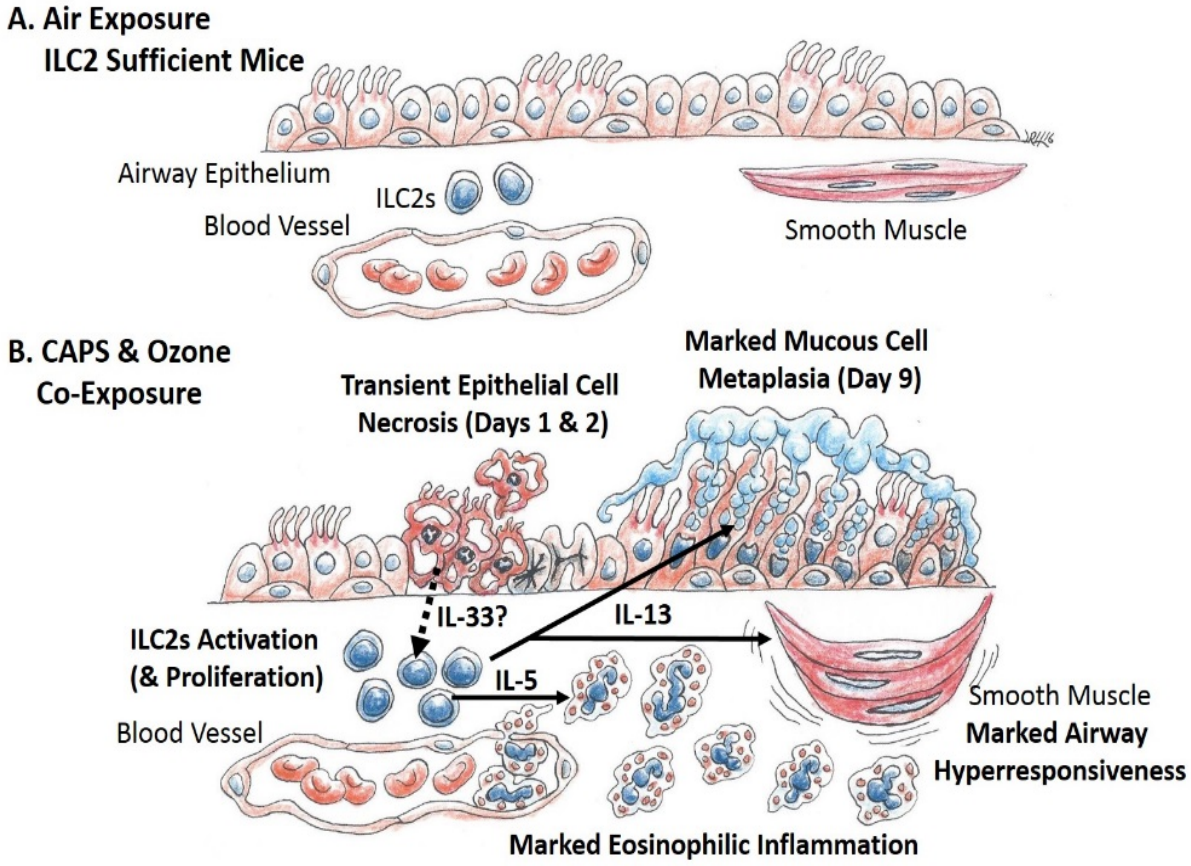
Summary and Conclusion

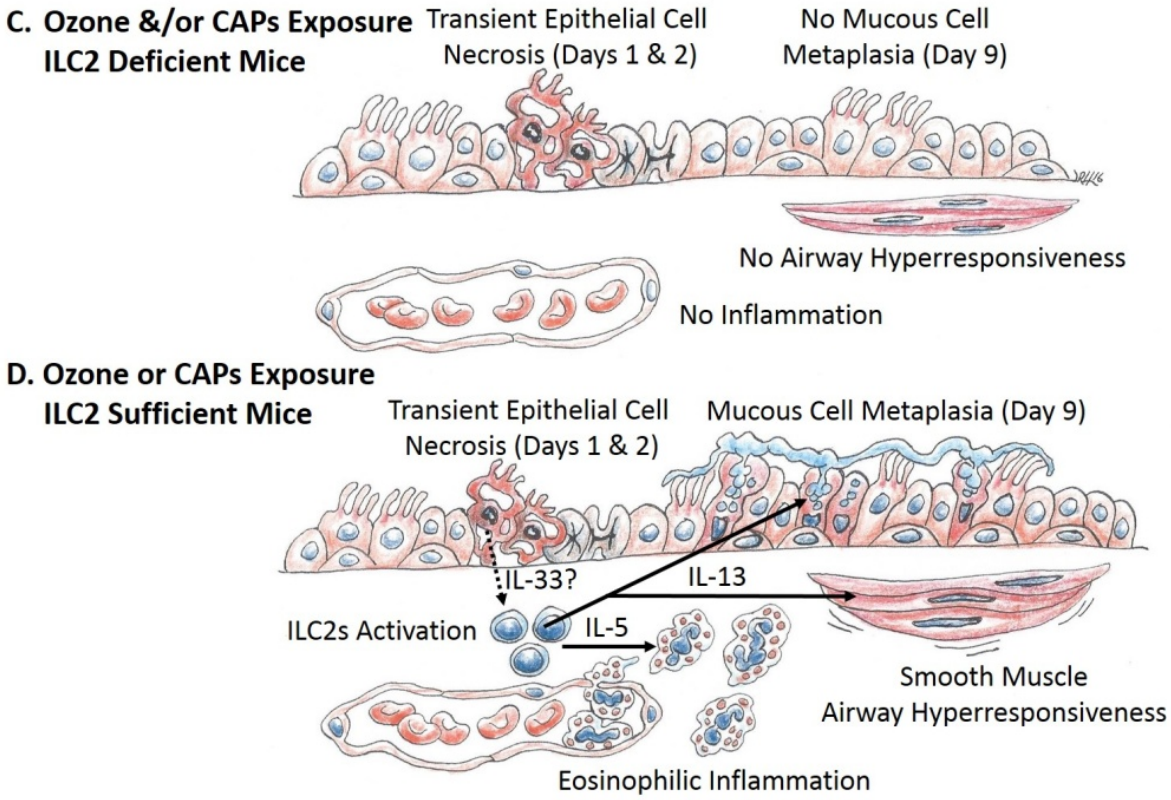
Mouse Strain	T & B Cells	ILCs	Ozone-Induced Lung Lesions (1-day Exposure)	Ozone-Induced Lung Lesions (9-day Exposure)
C57BL/6	+	+	+	+
$Rag2^{-/-}$	–	+	+	+
$Rag2^{-/-}Il2rg^{-/-}$	–	–	+	–

- These results indicate that ILC (most likely ILC2s) play a key role in the development of inflammatory and epithelial features of asthma caused by repeated, but not single, ozone exposure.
- This study provides a plausible biological paradigm for the epidemiologic association of ambient ozone exposure and the development of asthma in non-atopic Latino children.

Future Studies: Do other air pollutants and mixtures of air pollutants induce ILC2-dependent, non-atopic rhinitis and asthma?

Inhalation Exposures to Concentrated Fine Ambient Particles (CAPs; PM2.5) with and without Ozone





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