Comparison of Ethylene- and Ozone-Induced Nasal Toxicity: *A Similar Mode of Action?*

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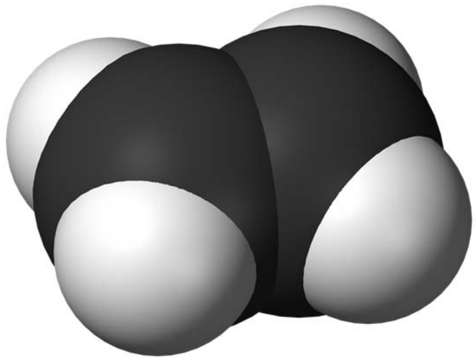
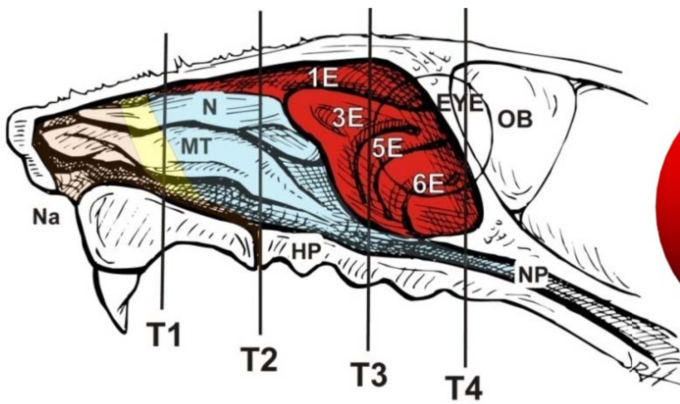
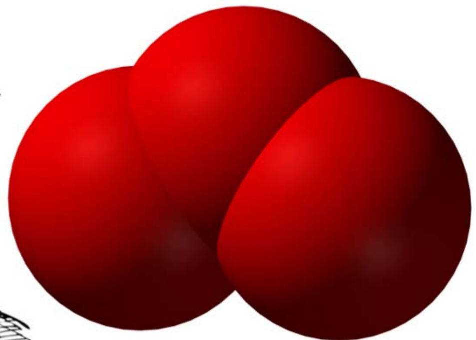






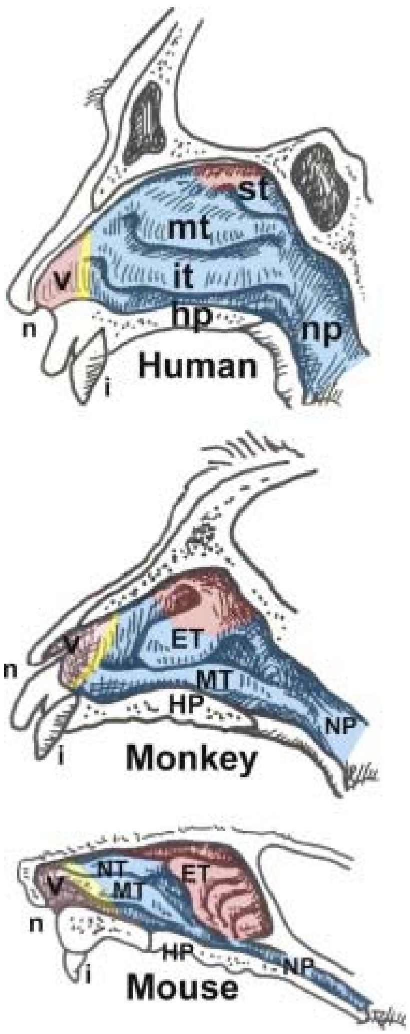
Understanding the Health Risks of Lower Olefins, 11/06/14, Austin, TX

Slides Prepared for the 2014 Symposium on Understanding the Health Risks of Lower Olefins

Outline

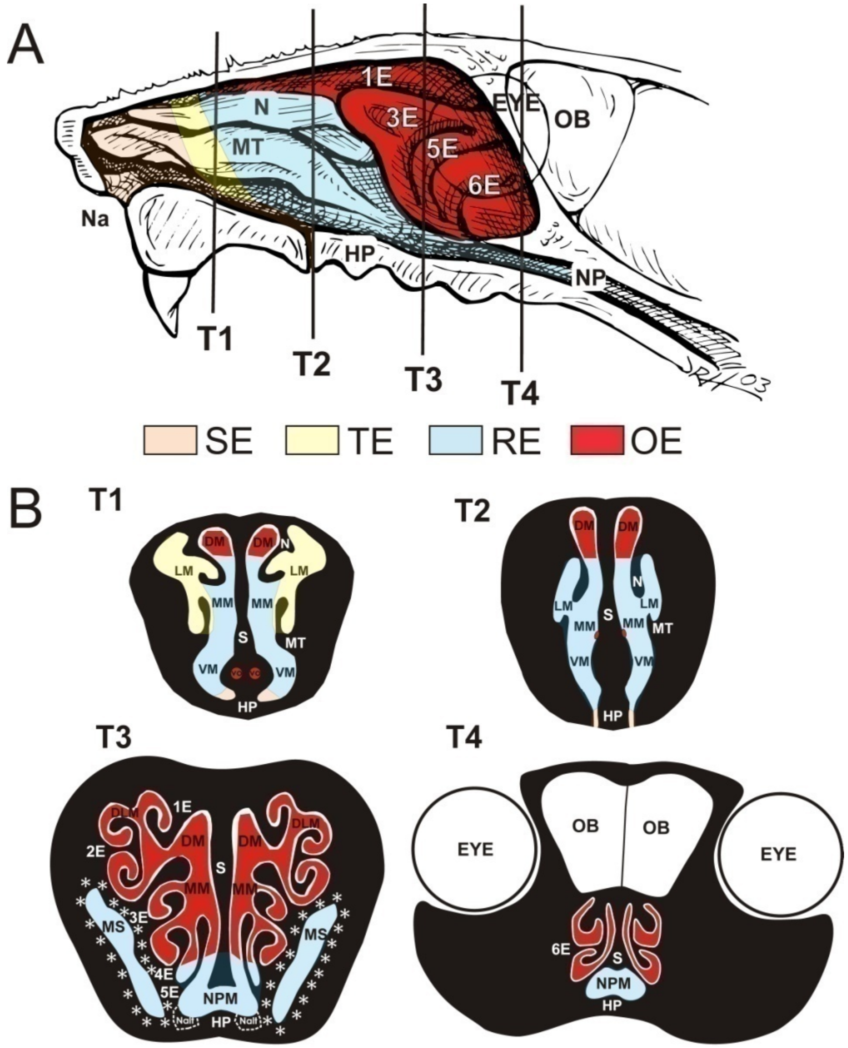
- Comparative nasal anatomy
- Nasal toxicity of ethylene and ozone
- Mode of action of ozone-induced nasal pathology
- Inhalation study to understand the mode of action of ethylene-induced nasal pathology
- Summary and questions

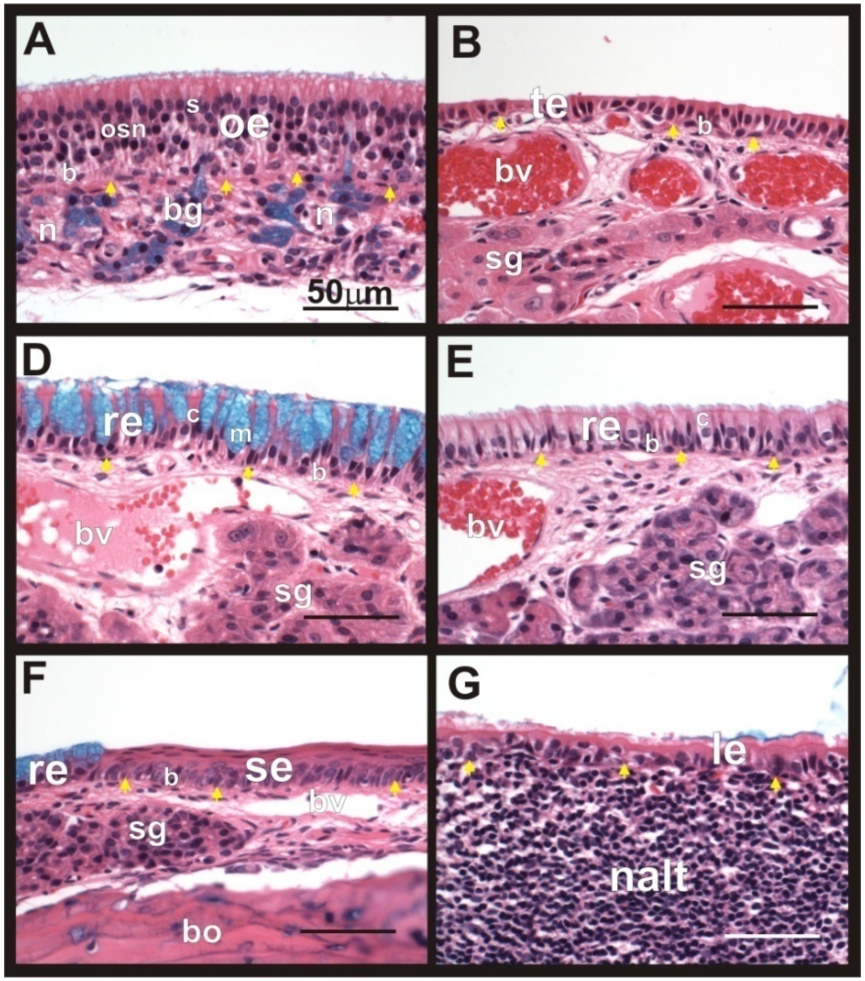
Comparative Nasal Airway Structure and Function



	Human	Monkey	Mouse
Volume (cm^3)	16	8	0.03
Turbinate Anatomy	Simple	Simple	Complex
Olfactory Epithelial Surface Area	Small <10%	Moderate 20-30%	Large 50%
Breathing	Oronasal	Oronasal	Nasal

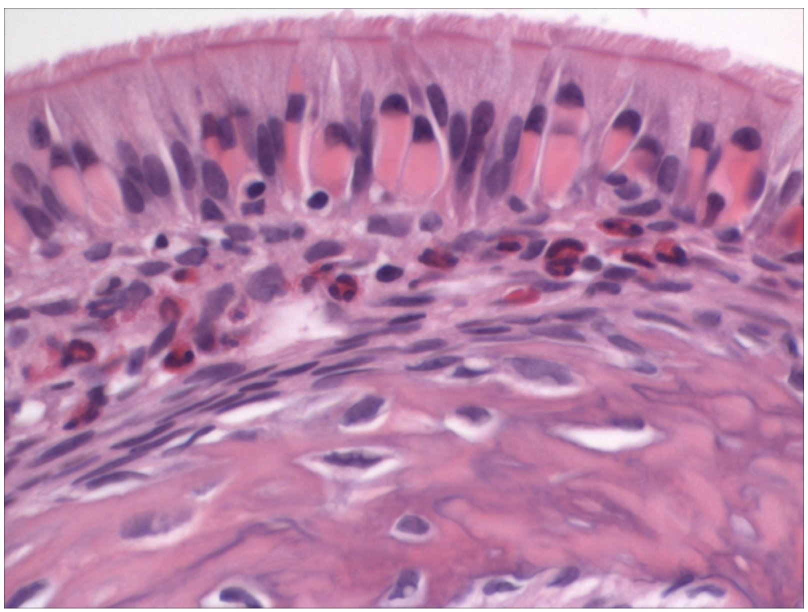
Rodent Nasal Airway Epithelium and Tissue Selection

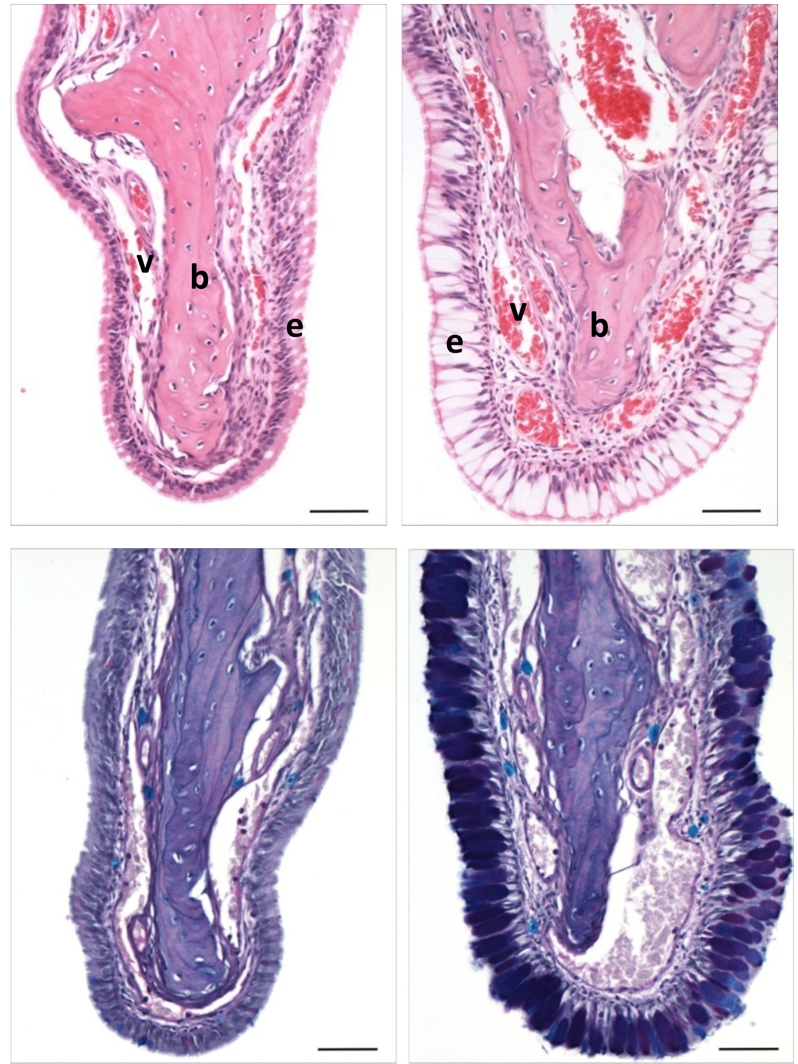




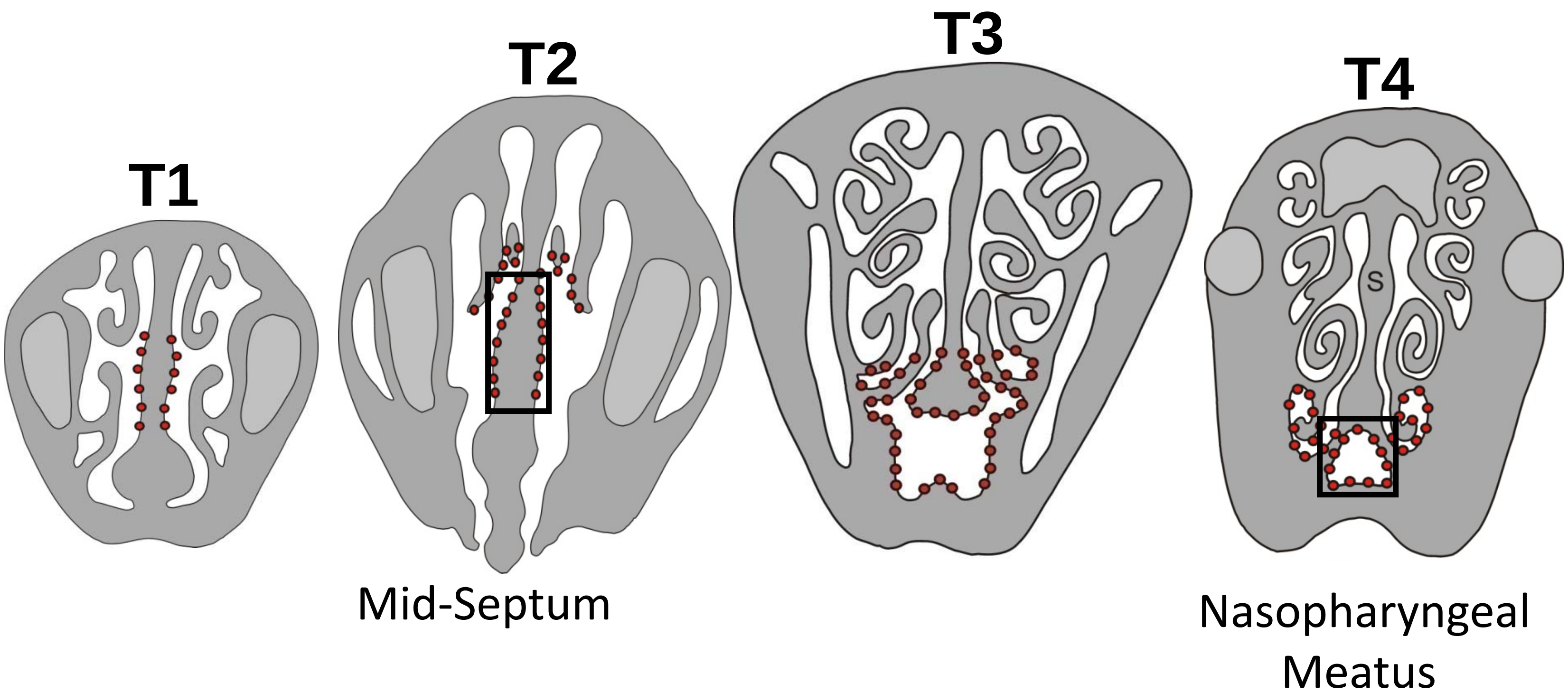
Ethylene-Induced Nasal Pathology

1. Eosinophilic Rhinitis
2. Increased Epithelial Mucus
3. Epithelial Hyalinosis
   (Ym1/Ym2 Protein)

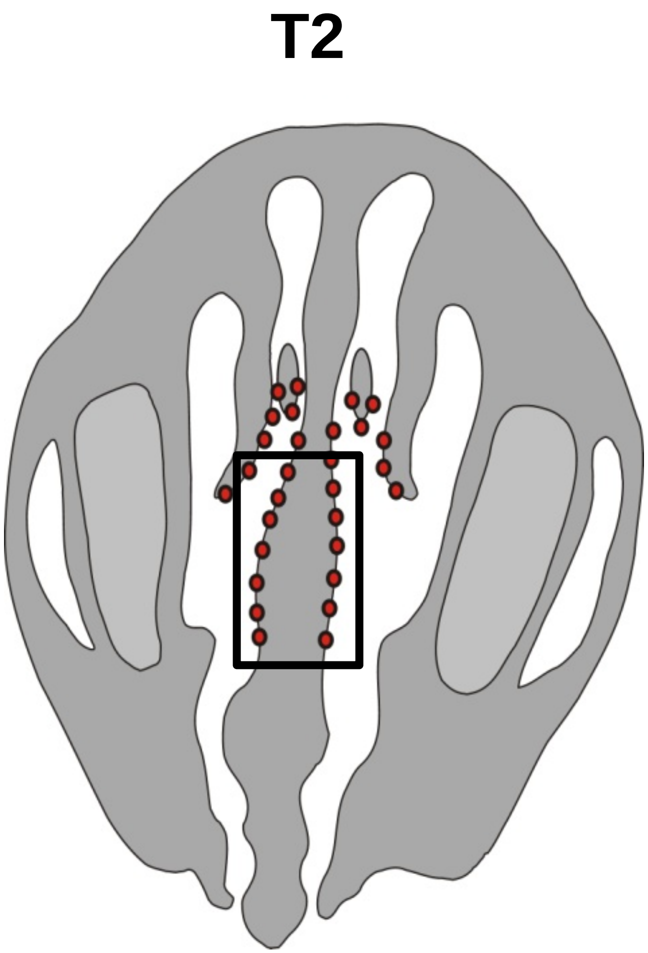


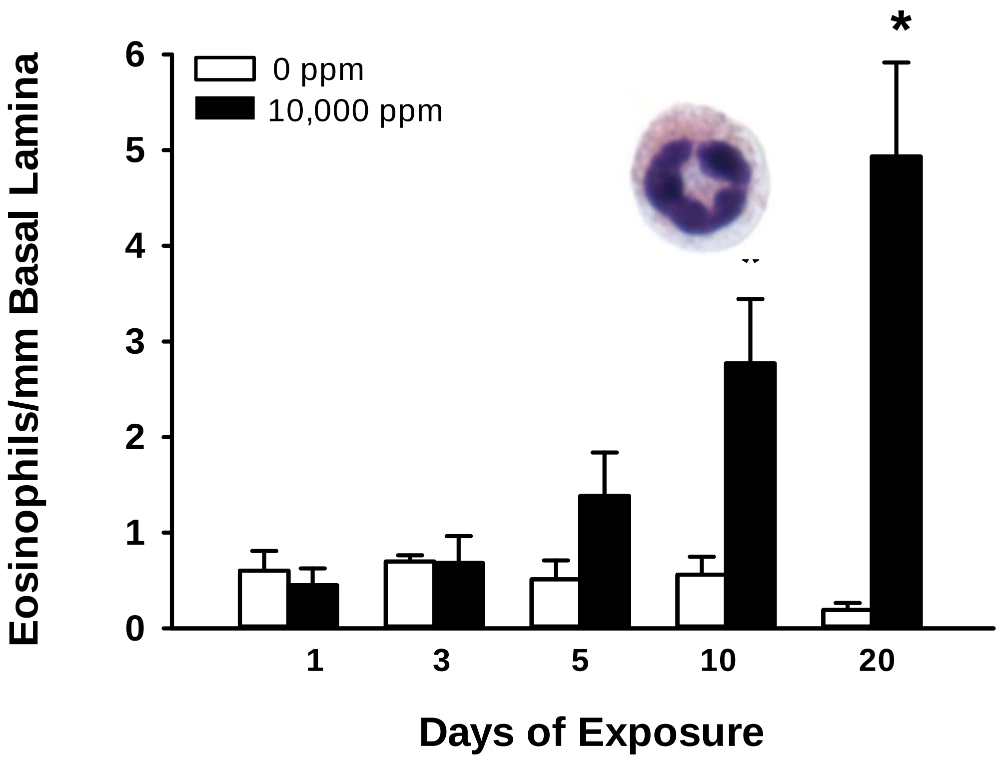


Distribution of Ethylene-Induced Nasal Inflammatory and Epithelial Lesions



Ethylene-Induced Eosinophilic Rhintis in F344 Rats

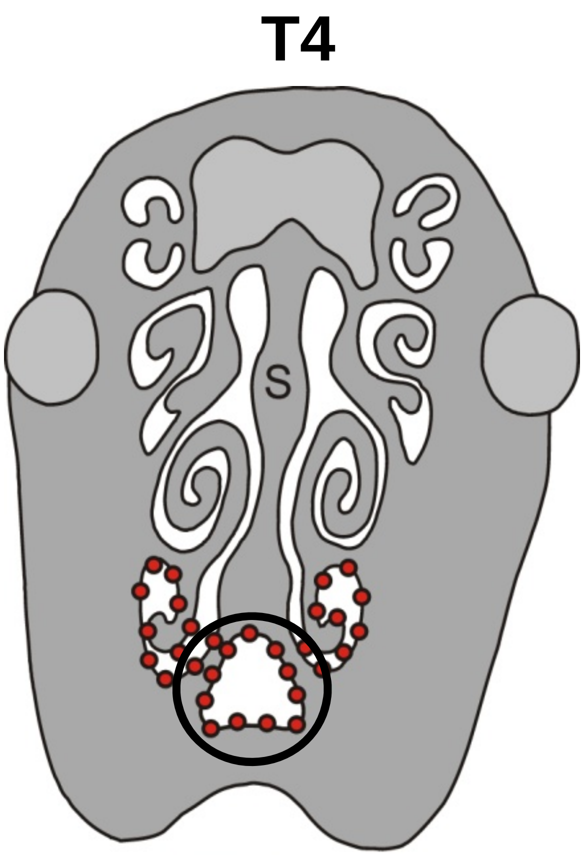


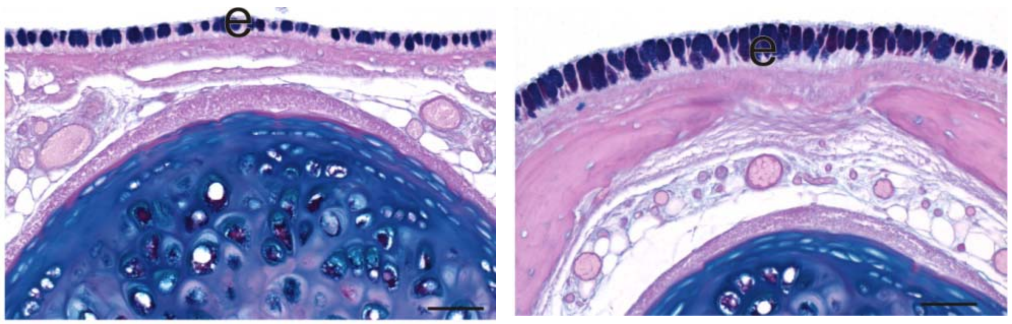


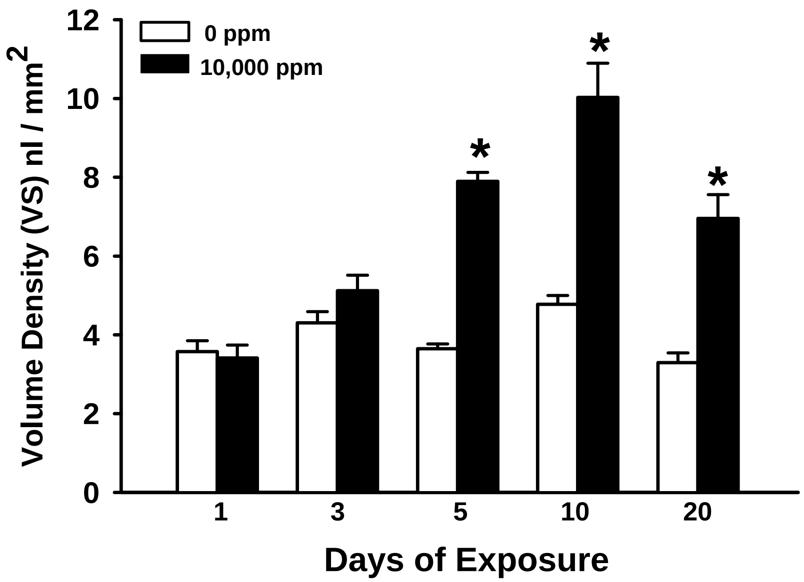
* = Significantly different from respective 0 ppm group, p ≤0.05

n = 8 rats/group

Ethylene-Induced Increase in Nasal Epithelial Mucosubstances in F344 Rats







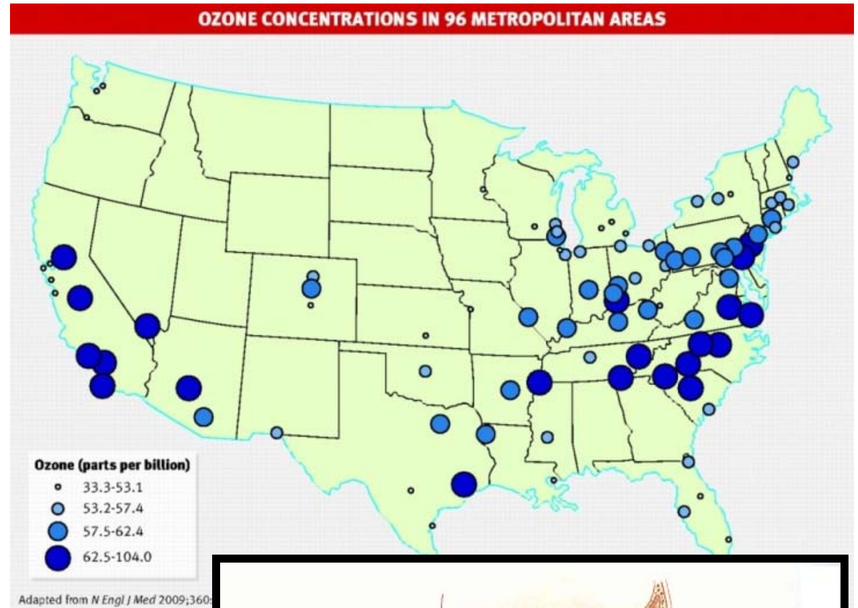
* = Significantly different from respective 0 ppm control group, $p \leq 0.05$

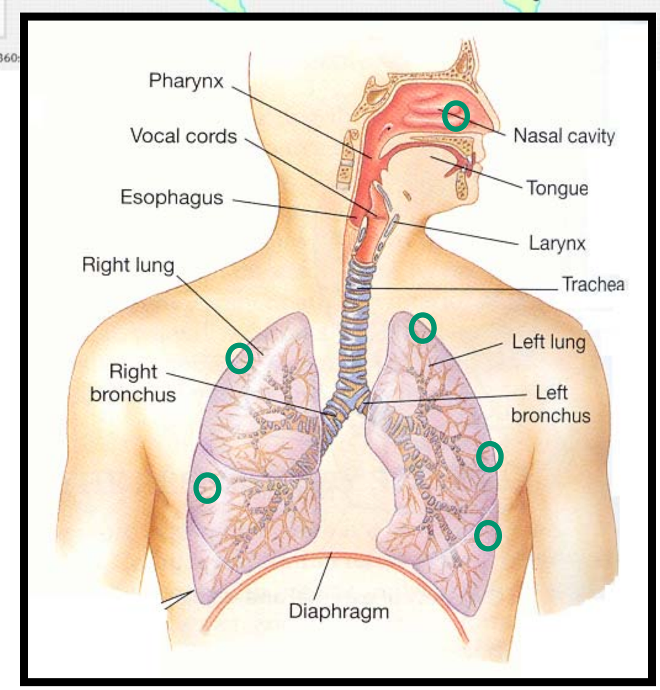
Comparison of Nasal Pathology in F344 Rats Exposed to Inhaled Toxicants

Toxicant	Rhinitis with Eosinophils	Increase in Epithelial Mucus	Epithelial Hyalinosis
Ethylene (10,000 ppm, 20 days)	Yes	Yes	Yes
Propylene (10,000 ppm, 20 days; Pottenger et al., 2007)	No	No	No
Ozone (0.5 ppm, 20 mo; Harkema et al., 1997)	Yes	Yes	Yes
Chlorine (2.5 ppm, 2 yr; Ibanes et al., 1996)	Yes	Yes	Yes

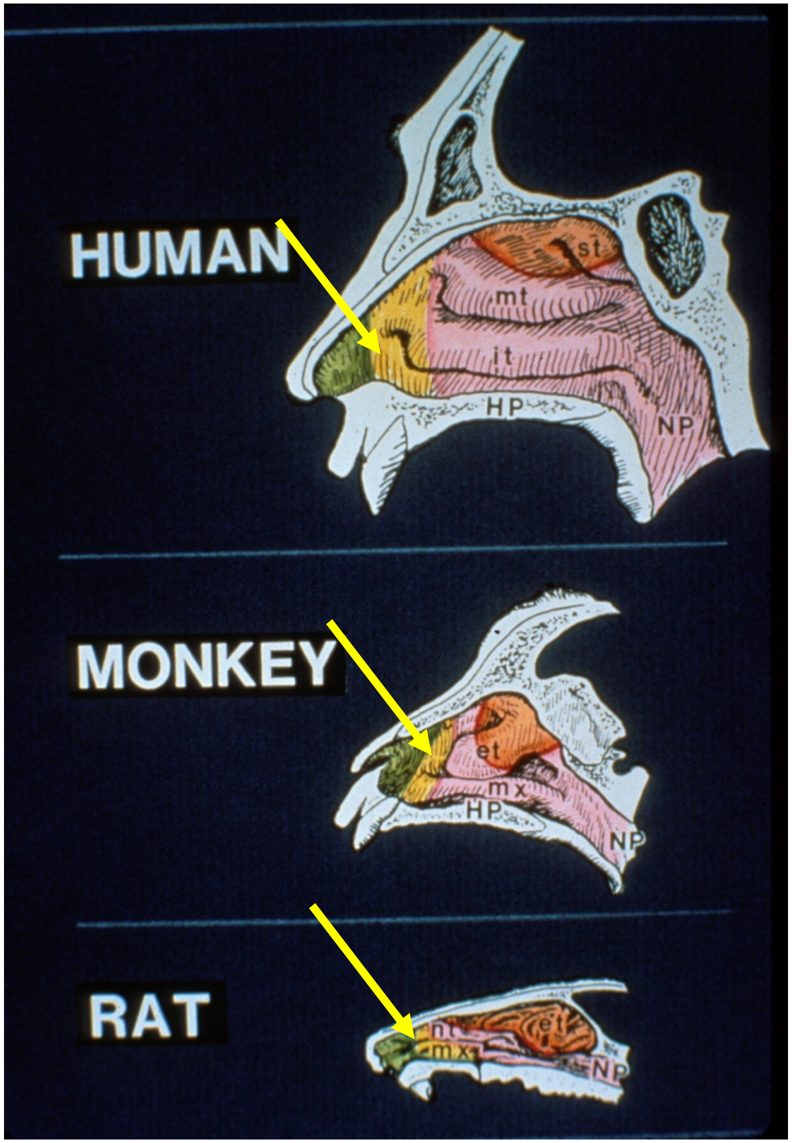
Ozone (O_3)

- One of the most reactive chemicals
- Secondary gaseous air pollutant in photochemical smog
- 131 million people (45% of the U.S. population) live in communities where average ambient concentrations exceed the NAAQS
- Respiratory toxicant causing airway inflammation and remodeling
- Long-term exposure causes an increase in mortality





Comparative Nasal Toxicity of Ozone



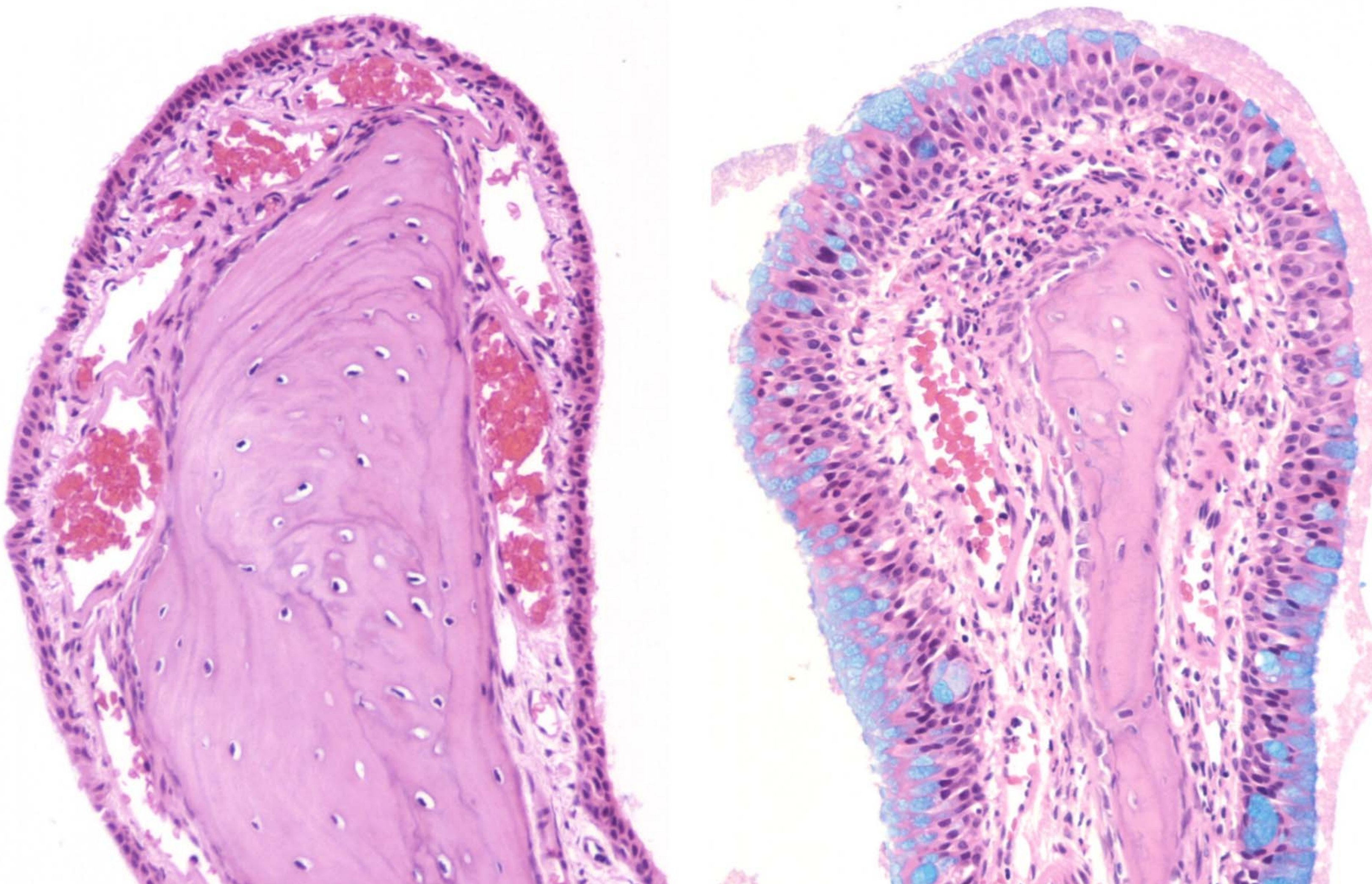
Calderon-Garciduenas et al. *Am. J. Pathol.* 140: 225-32, 1992

Harkema et al. *Am. J. Pathol.* 127:90-96, 1987

Harkema et al. *Am. J. Pathol.* 128:29-44, 1987

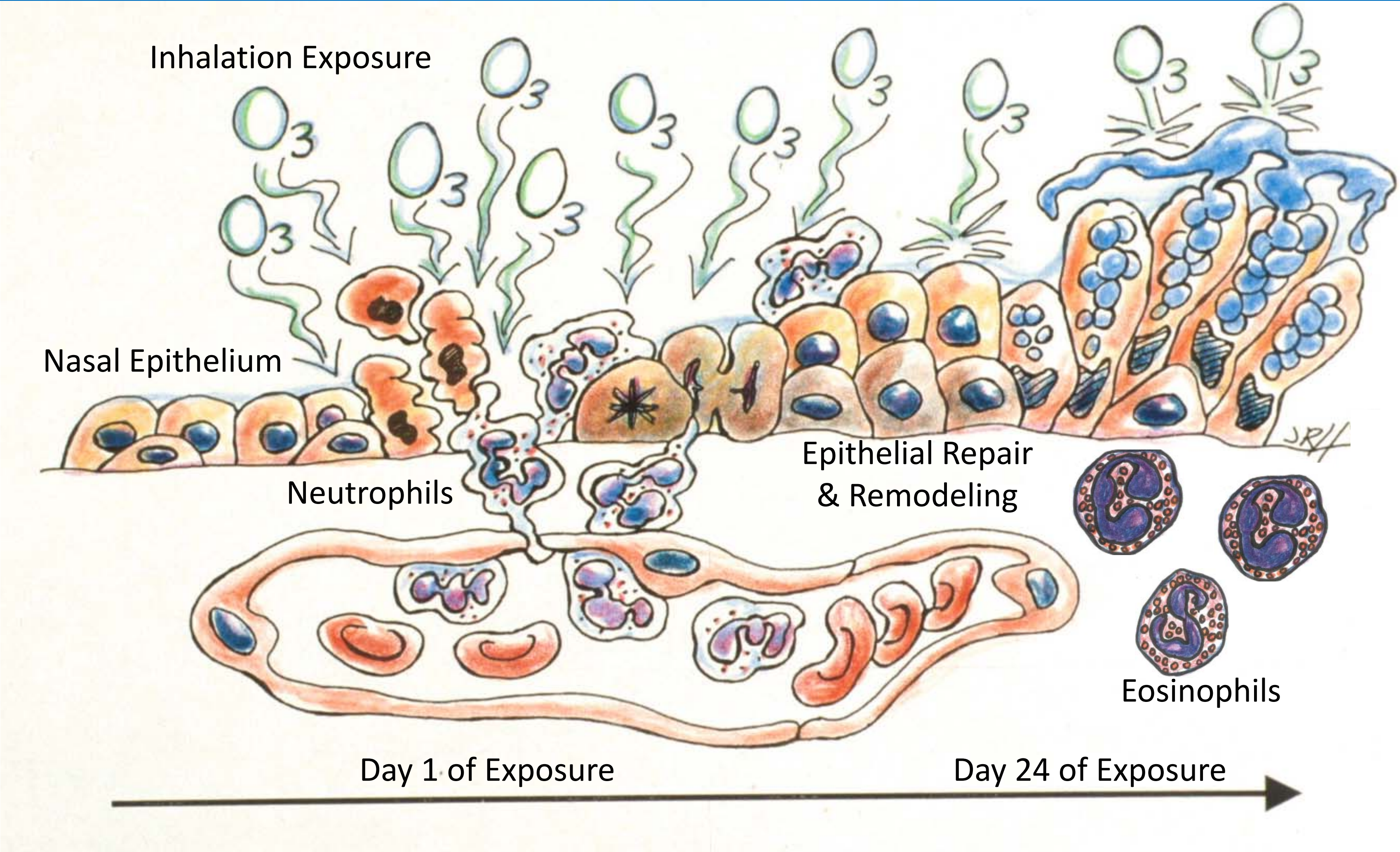
Harkema et al. *Toxicol. Pathol. 17: 525-535,* 1989

O3-Induced Remodeling of Maxilloturbinate in Rat



Filtered Air (0 ppm O3)   13 wks, 5 days/wk   O3 (0.5 ppm)

Eosinophilic Rhinitis and Nasal Epithelial Remodeling in Mice Exposed to Ozone



Injury → Necrosis → Neutrophils → Eosinophils & Mucous Cell Metaplasia

Temporal Cell and Cytokine Responses in the Nasal Airway of Mice Exposed to Ozone

- C57BL/6 male mice
- 0 or 0.5 ppm ozone exposure
- 4h/day for 1, 2, 4, 9 or 24 consecutive weekdays
- Nasal histopathology and morphometry
- RT-PCR (epithelial and inflammatory gene expression)
- Clustering of gene expression and phenotype changes with time of exposure

Ong Chee Bing, BVS, MS

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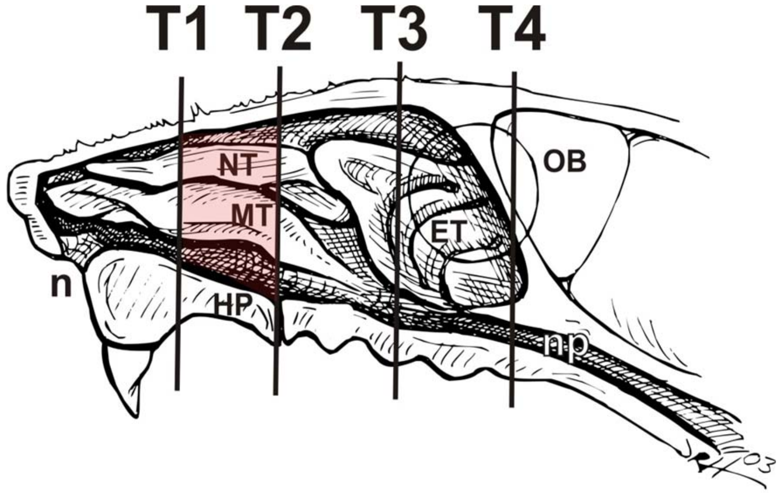
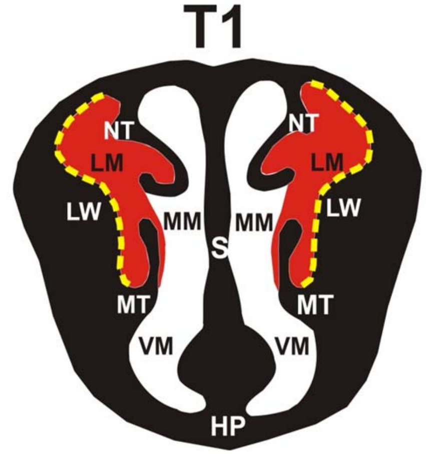
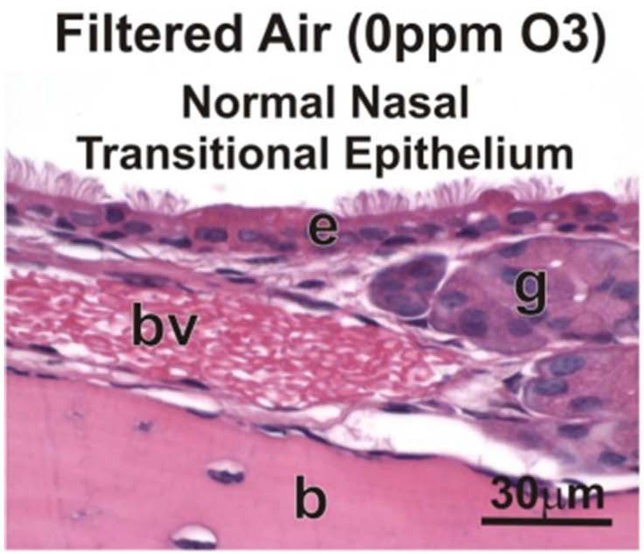
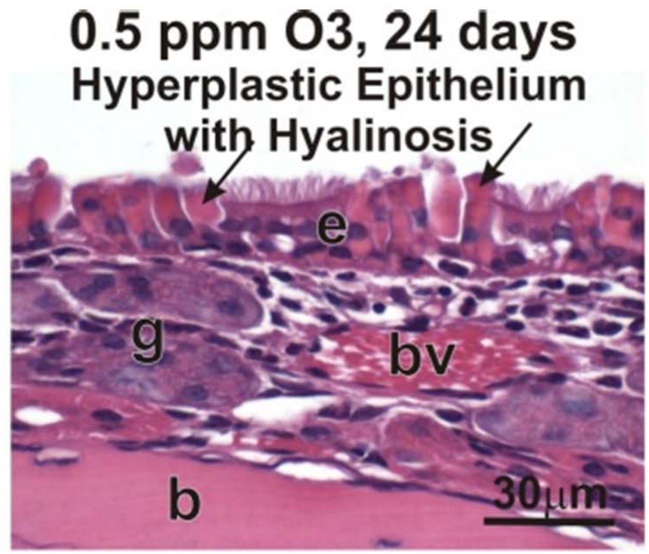
Ozone-Induced Nasal Pathology Restricted to the Proximal Lateral Wall

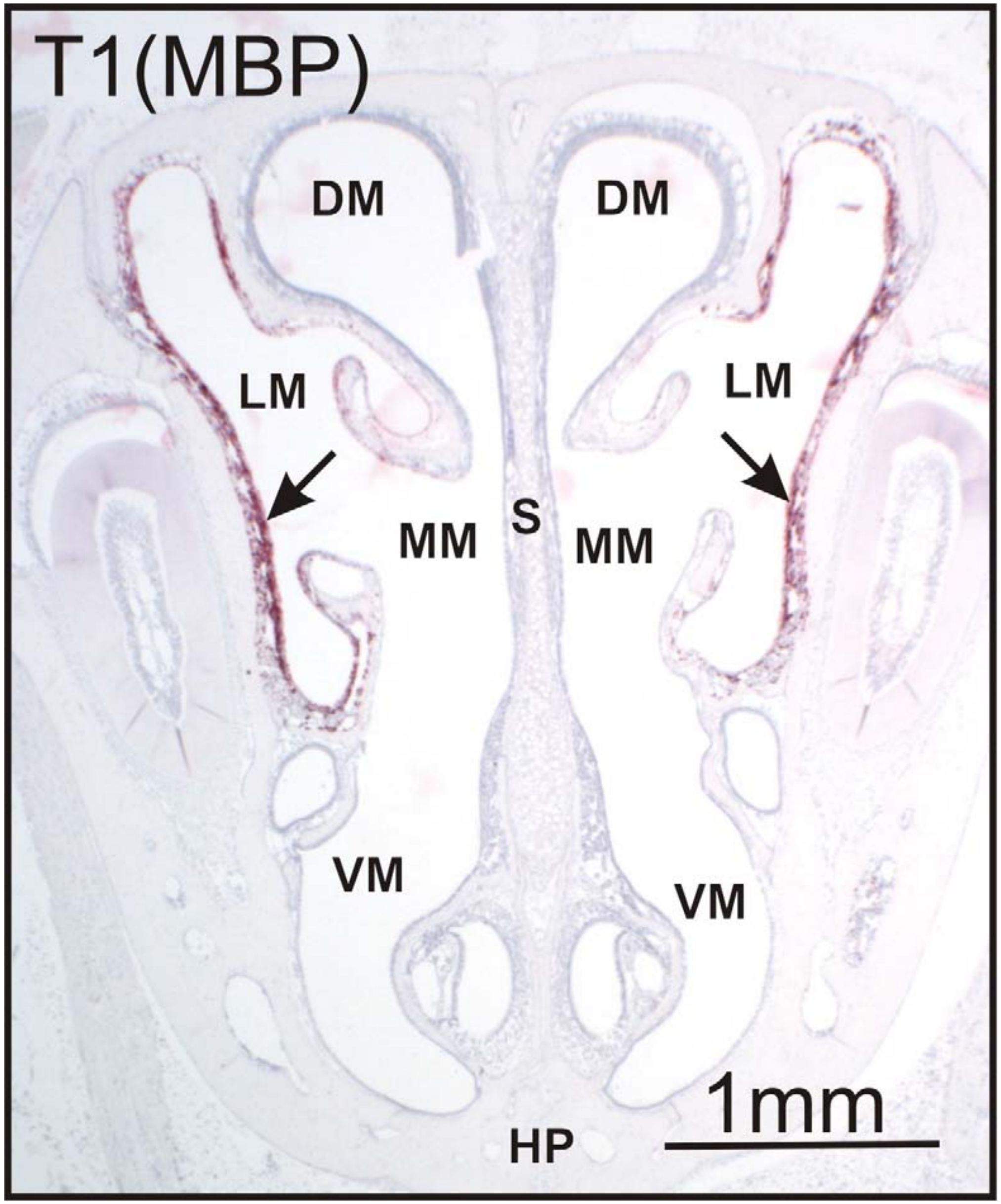


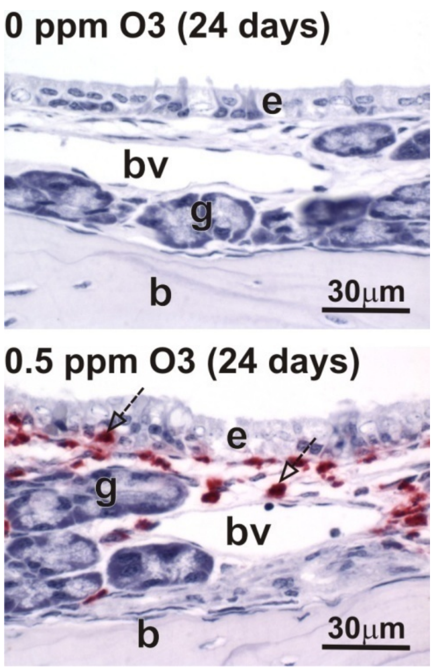


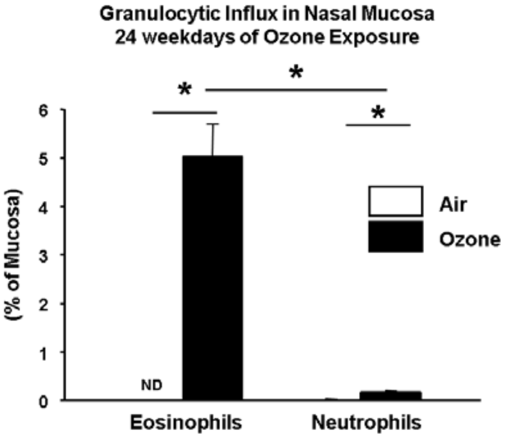




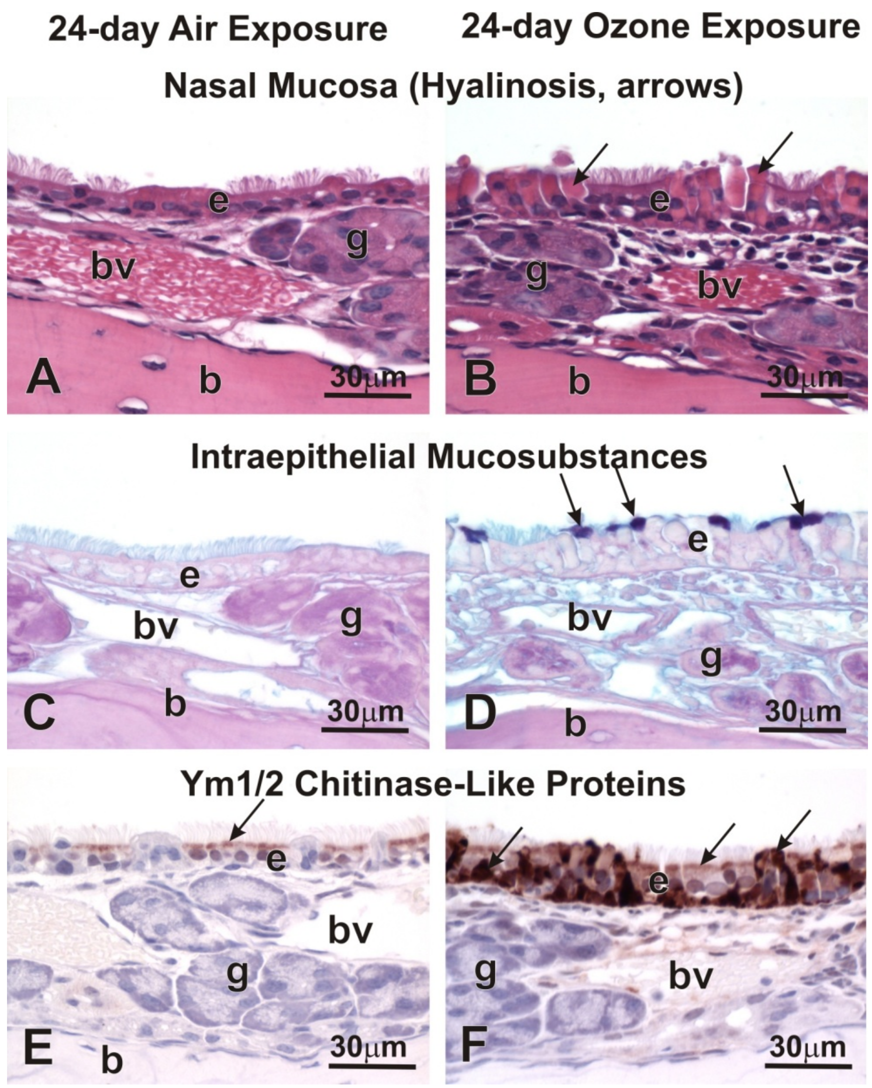
Major Basic Protein-laden Eosinophils

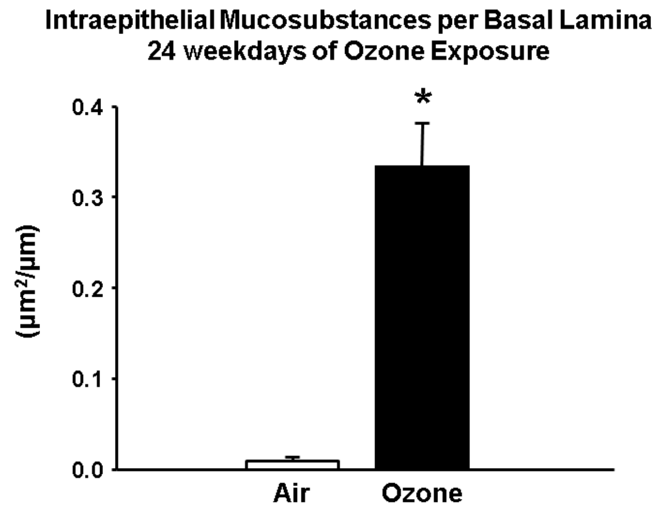


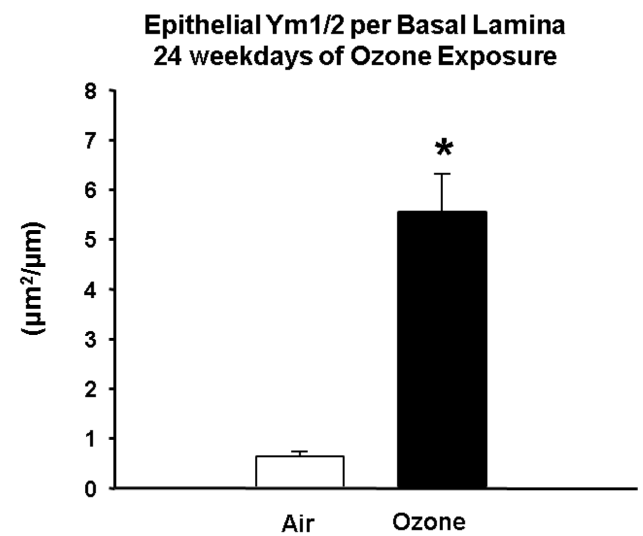




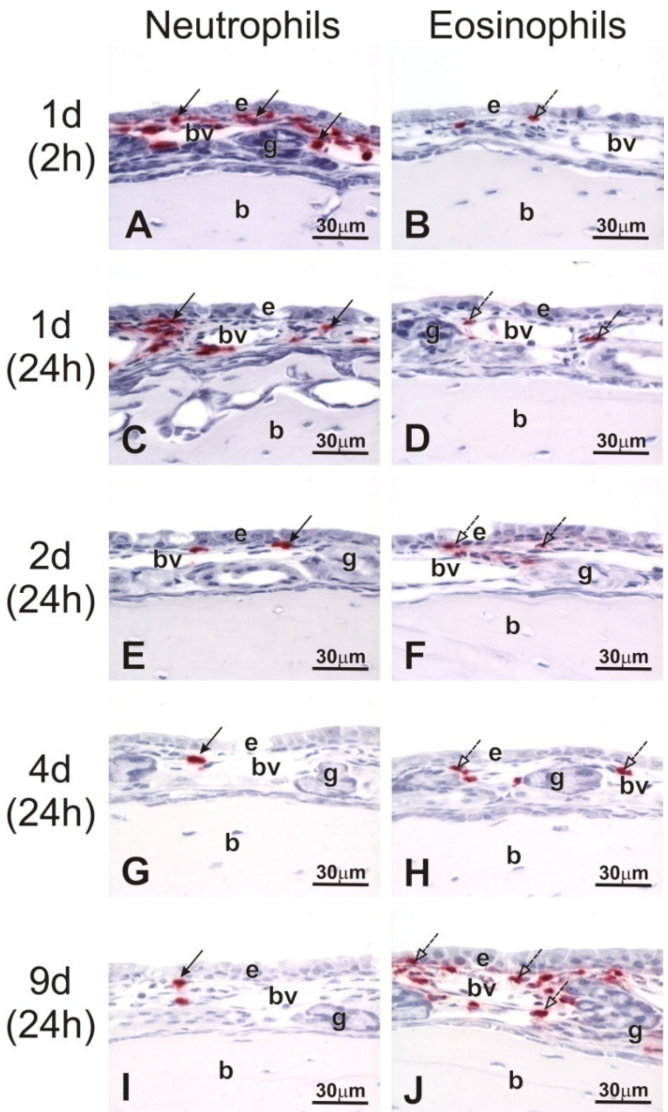
Nasal Epithelial Remodeling in Mice Exposed to Ozone



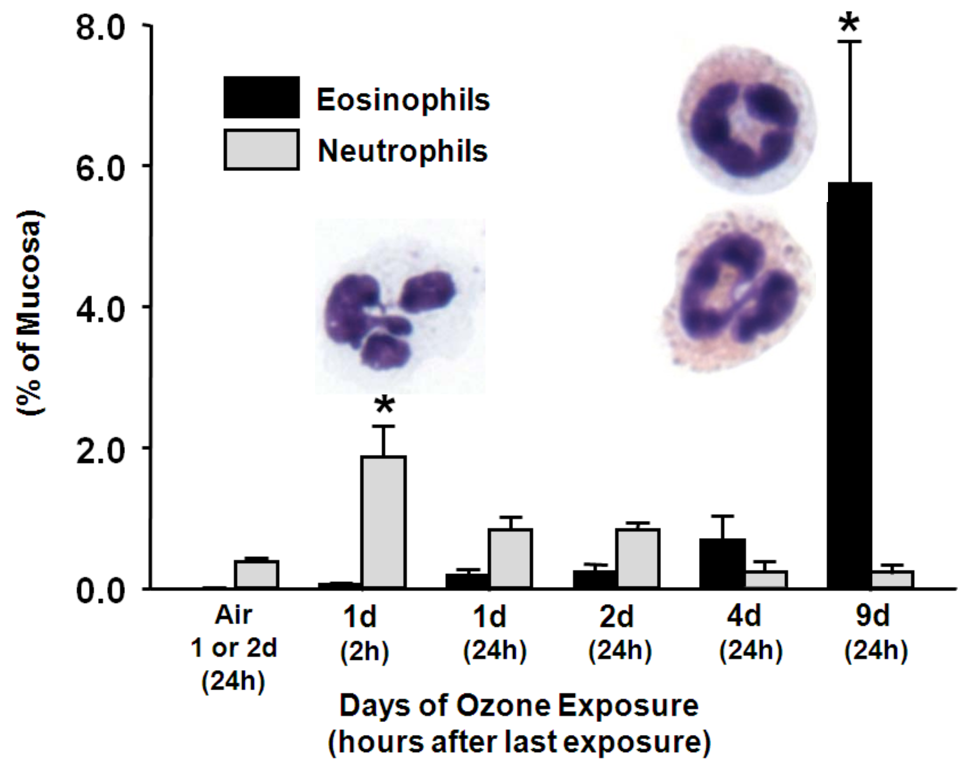




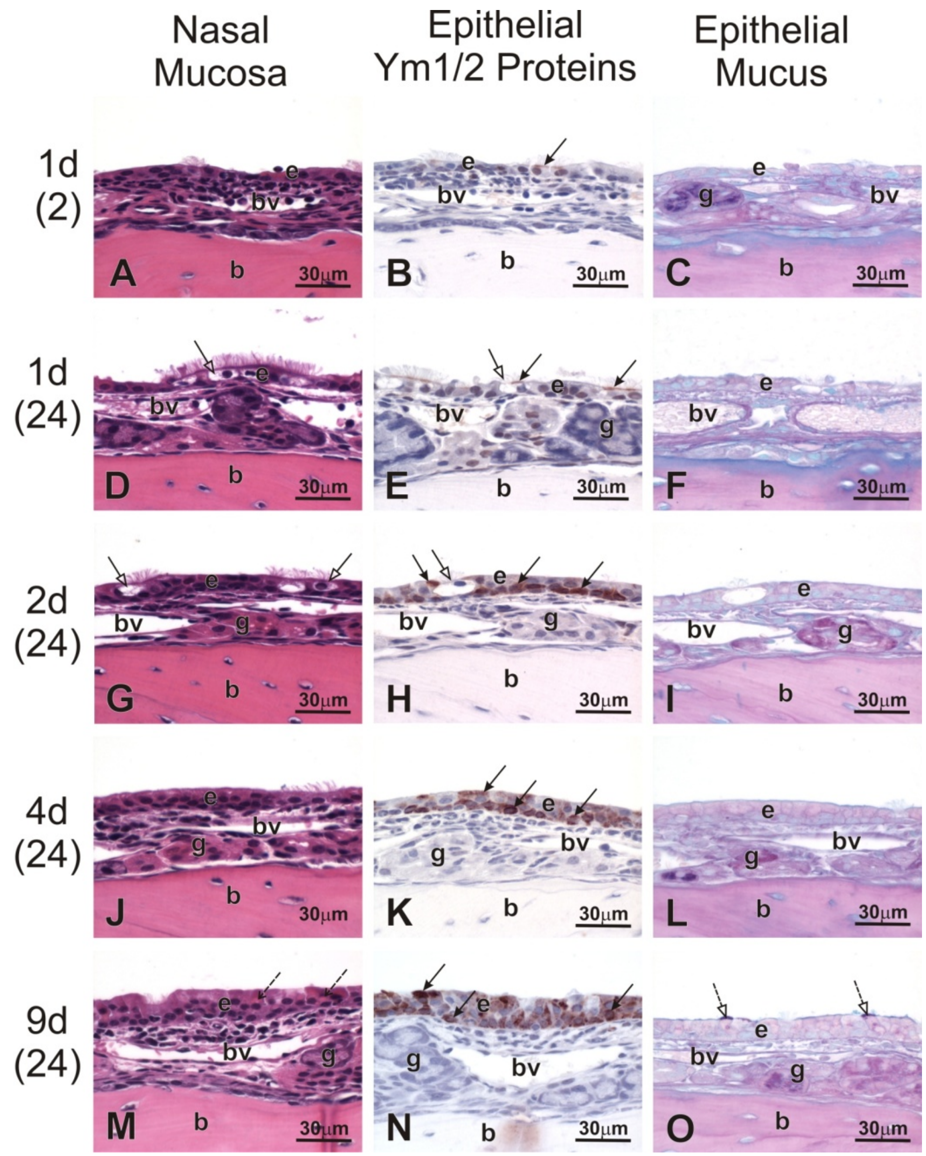
Temporal Changes in Granulocytic Influx with Repeated Ozone Exposure (1-9 days; 4h/day)



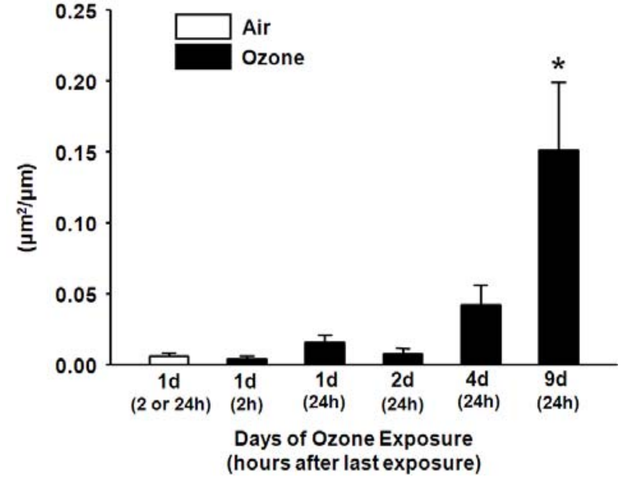
Density of Granulocytes in Nasal Mucosa



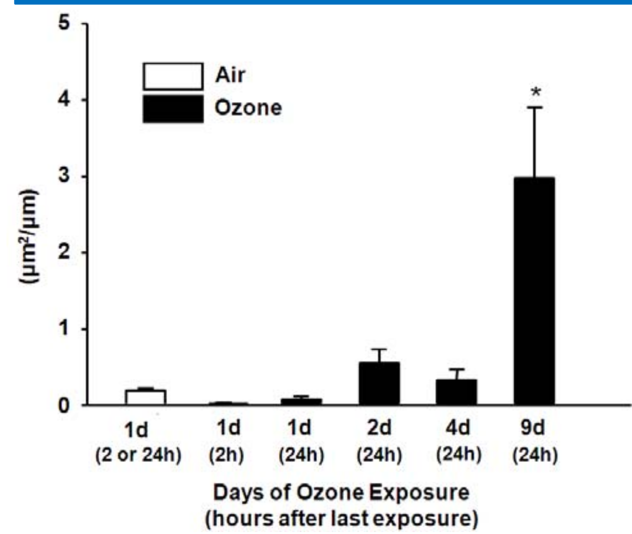
Temporal Changes in Nasal Epithelium with Repeated Ozone Exposure (1-9 days; 4h/day)



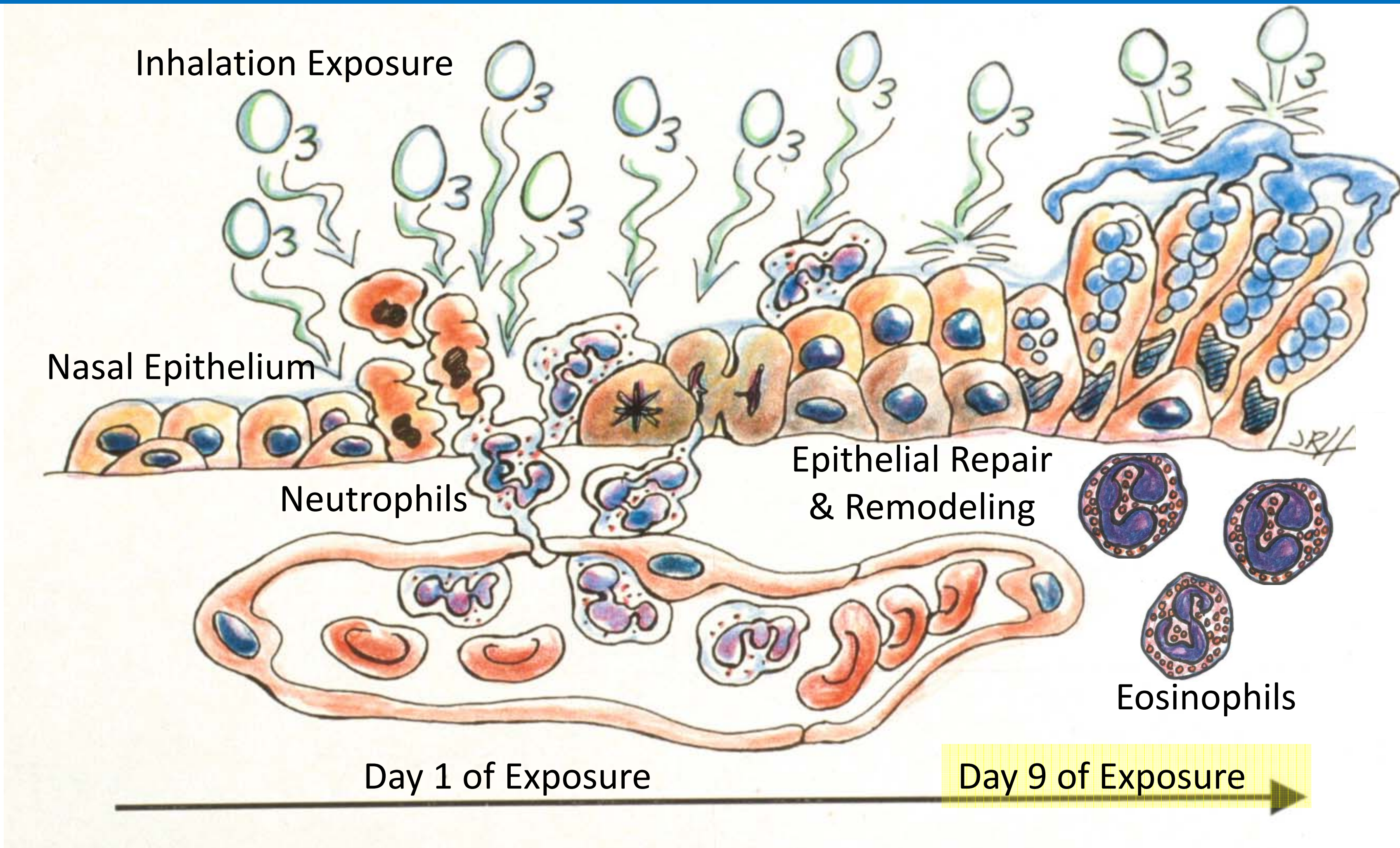
Epithelial Mucosubstances



Epithelial Ym1/Ym2 Protein



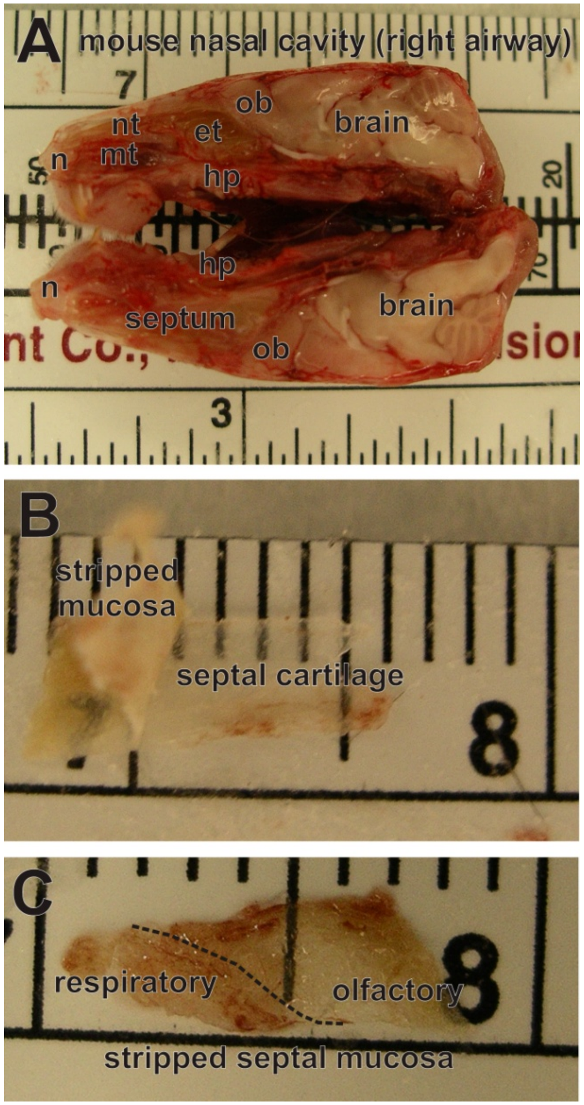
Eosinophilic Rhinitis and Nasal Epithelial Remodeling in Mice Exposed to Ozone



Injury → Necrosis → Neutrophils → Eosinophils & Mucous Cell Metaplasia

Selected nasal mucosal tissues for gene expression analyses (PCR-arrays, qRT-PCR)

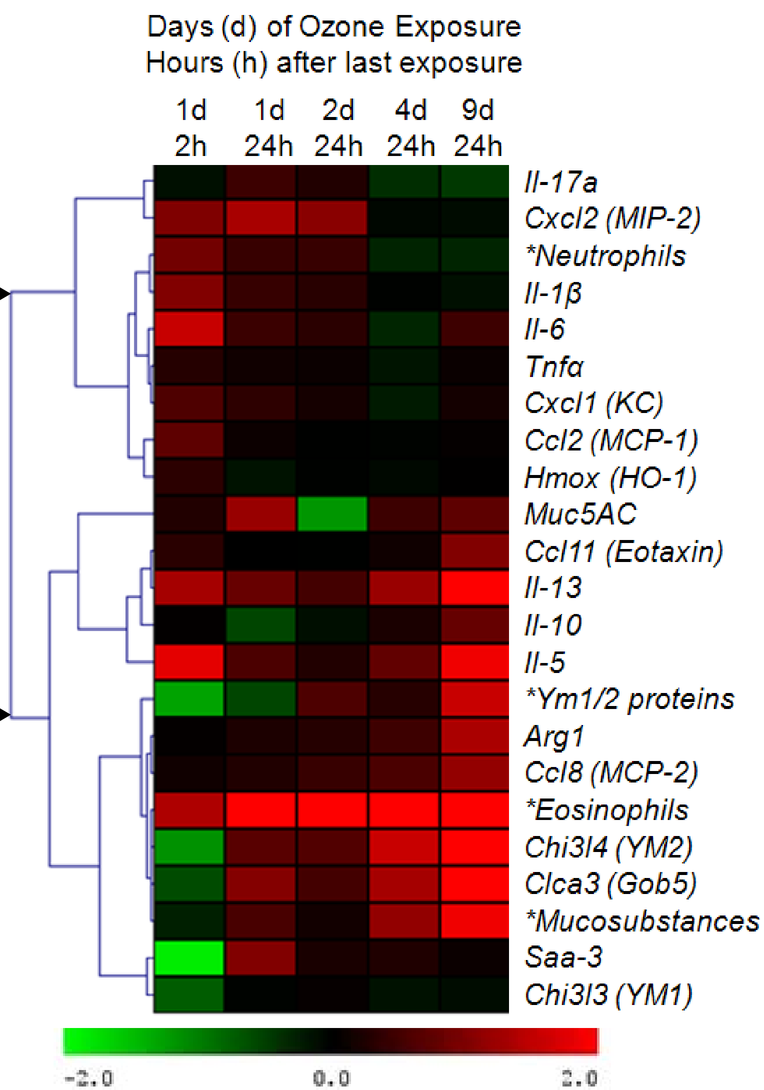
- Mice sacrificed at designated times post-exposure
- Nasal cavities split in half
- Two halves immediately immersed in RNAlater® solution
- Nasal mucosal tissues microdissected from airways
- RNA extracted from site-selected nasal mucosal tissues
- Conducted PCR-arrays (pooled cDNA by group) and qRT-PCR assays (cDNA from individual rats)



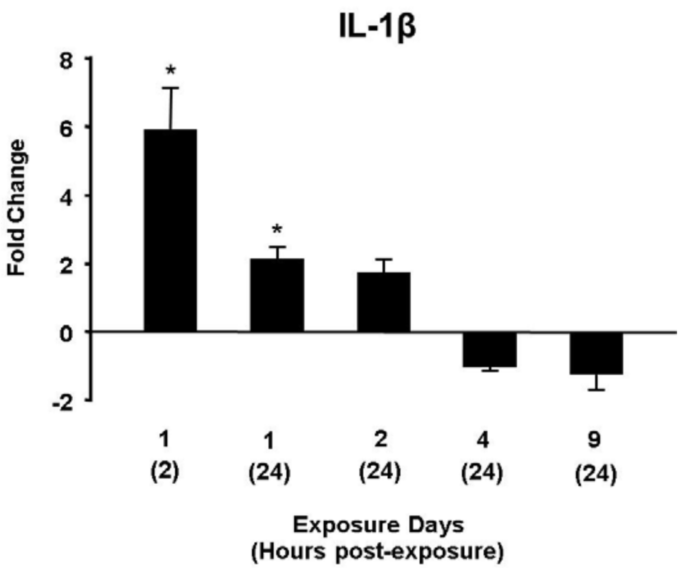
Unsupervised Hierarchical Clustering of Temporal Changes in O3-Induced Gene Expression and Morphometric *Phenotypes in the Nasal Mucosa

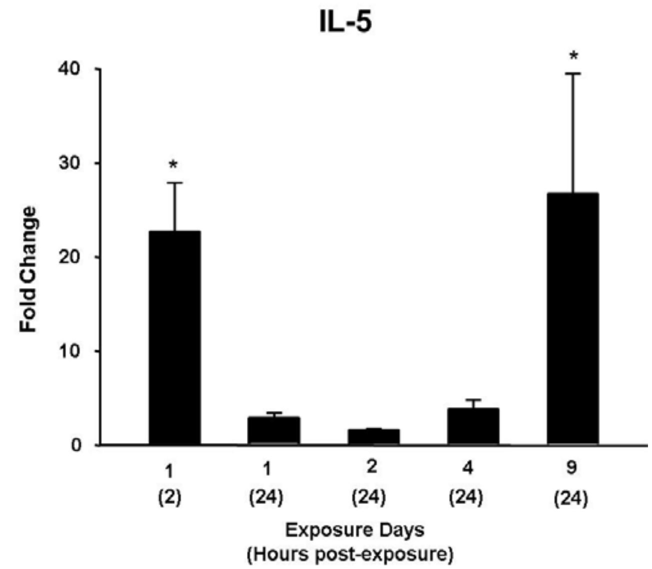
Th1 Inflammatory Profile →

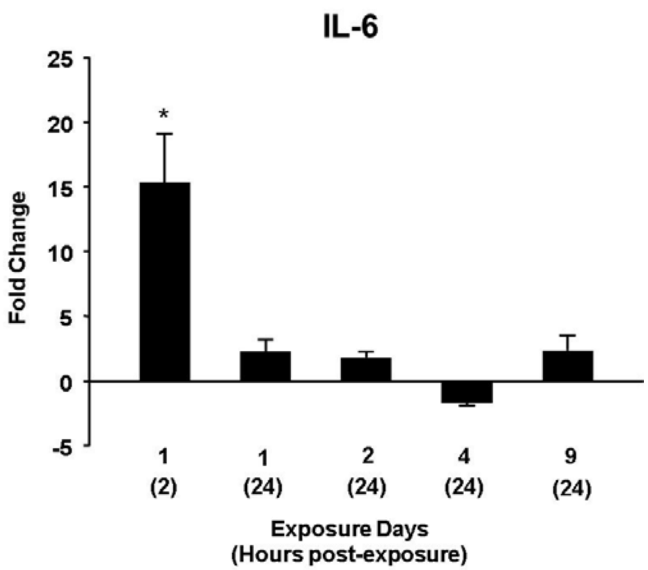
Th2 Inflammatory Profile →

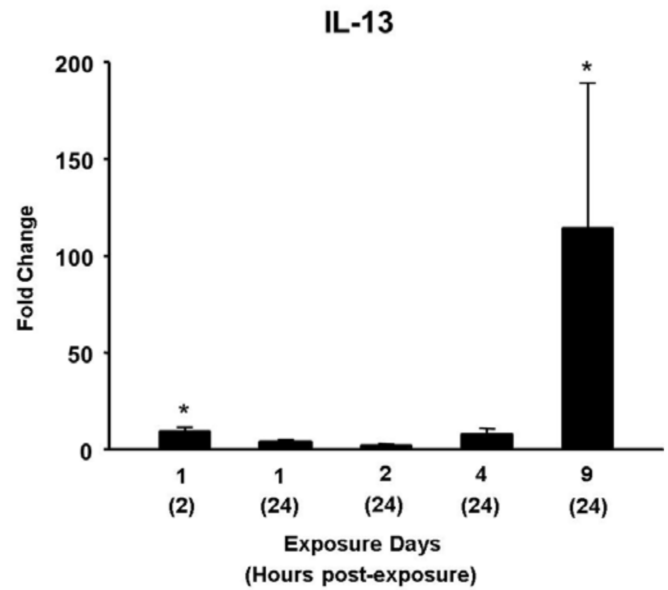


Temporal Fold Changes in Th1 and Th2 Cytokine Gene Expression









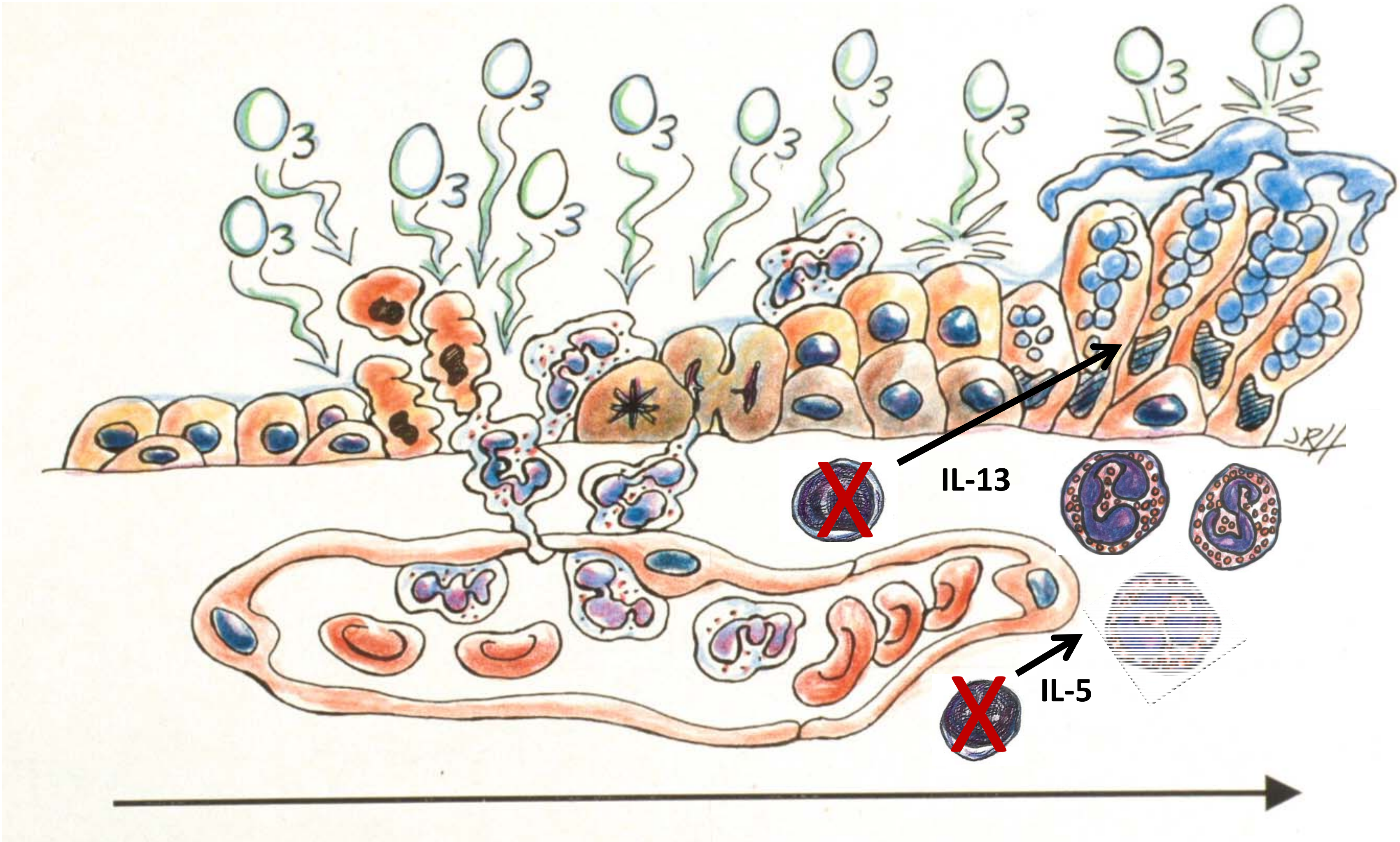
Study Summary and Conclusions

- Repeated, episodic ozone exposures in mice induce Th2 cytokine overexpression, eosinophilic rhinitis and increased epithelial chitinase-like proteins (Ym1/Ym2).

- These results suggest an etiologic role of ambient ozone in the development of nonallergic eosinophilic rhinitis.

- Our animal study gives biologic plausibility to epidemiologic associations between ozone exposure and eosinophilic rhinitis and systemic eosinophil cationic proteins in children (Frischer et al. 2001, 1993)

What is the Role of Lymphoid Cells in Eosinophilic Rhinitis & Nasal Epithelial Remodeling in Mice Exposed to Ozone?



Rag 2-/- Gamma C-/- Mice

- Cross of common cytokine receptor gamma chain (γ c) KO mouse with Rag2 (recombinase activating gene 2)-deficient mice
- Double KO mice lack T cells, B cells, NK cells, and type 2 innate lymphoid cells (ILCs) – Lymphoid cell-deficient animals
- Mice are useful in combination with parental Rag2 KO mice for sorting out the role of ILCs

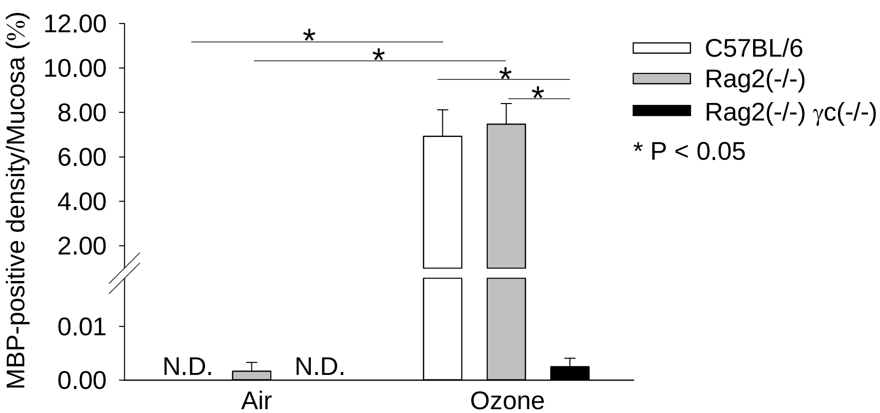
Rag 2- /- Mice

- Contains a disruption of the recombination activating gene 2 (Rag2)
- Homozygous mice exhibit total inability to initiate V(D)J rearrangement and fail to generate mature T or B lymphocytes
- Do have type 2 innate lymphoid cells

Ozone-Induced Eosinophilic Rhinitis is Dependent on Innate Lymphoid Cells

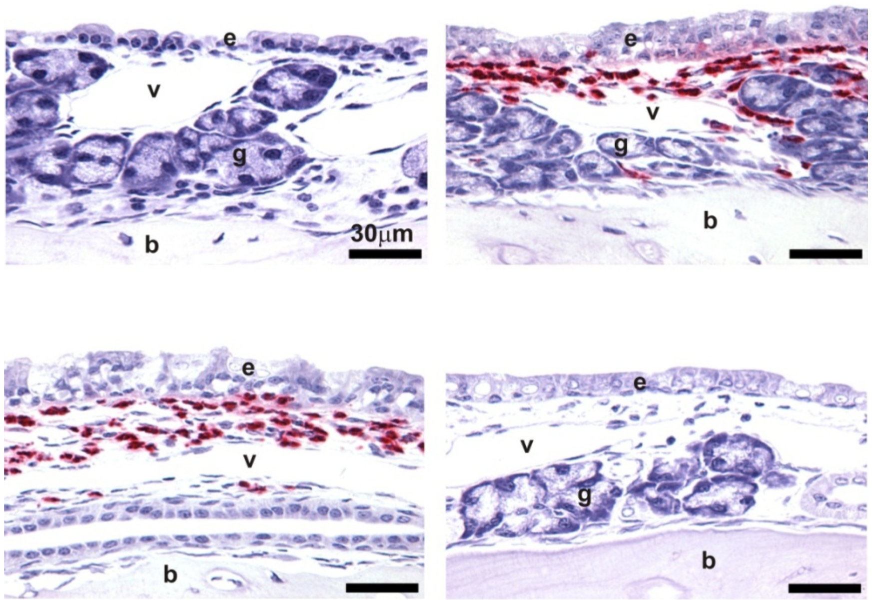
Mouse Strain	Filtered Air	0.8 ppm O3
C57BL/6	6	6
Rag2(-/-)	6	6
Rag2(-/-)ɣc(-/-)	6	6

Density of Eosinophils in the Nasal Mucosa



Air Exposed
T, B & ILCs Sufficient
C57BL/6

O3 Exposed
T, B, & ILCs Sufficient
C57BL/6



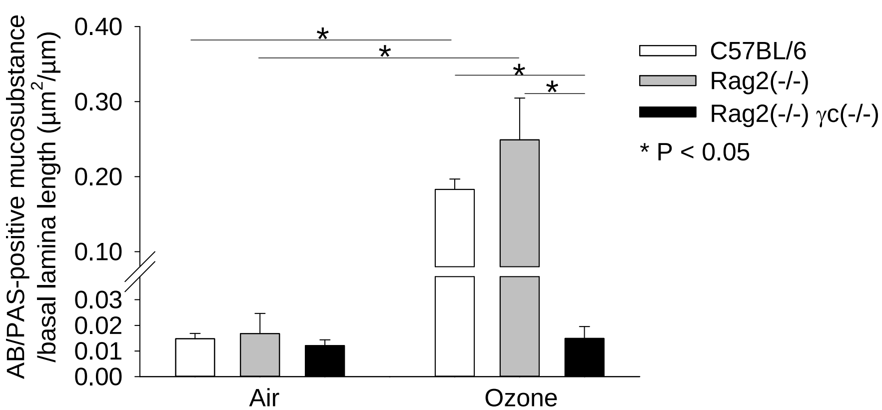
O3 Exposed
T & B Cells Depleted
ILCs Sufficient
Rag2(-/-)

O3 Exposed
T, B, & ILCs Depleted
Rag2(-/-)γc(-/-)

Ozone-Induced Mucous Cell Metaplasia is Dependent on Innate Lymphoid Cells

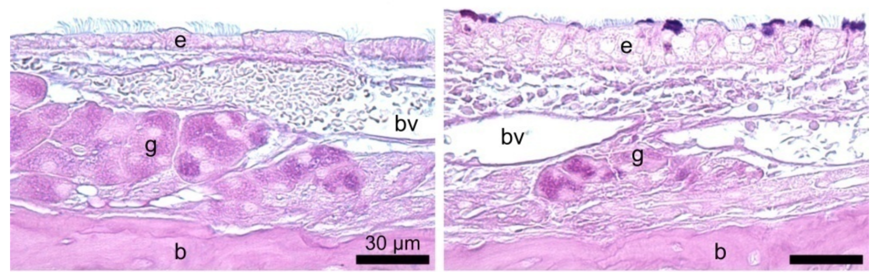
Mouse Strain	Filtered Air	0.8 ppm O3
C57BL/6	6	6
Rag2(-/-)	6	6
Rag2(-/-)ɣc(-/-)	6	6

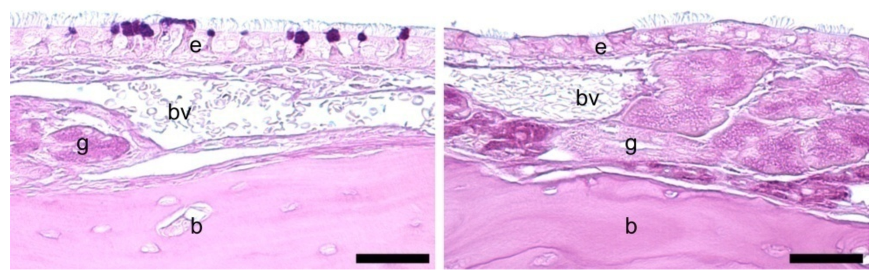
Volume Density of Epithelial Mucosubstances



Air Exposed
T, B & ILCs Sufficient
C57BL/6

O3 Exposed
T, B, & ILCs Sufficient
C57BL/6





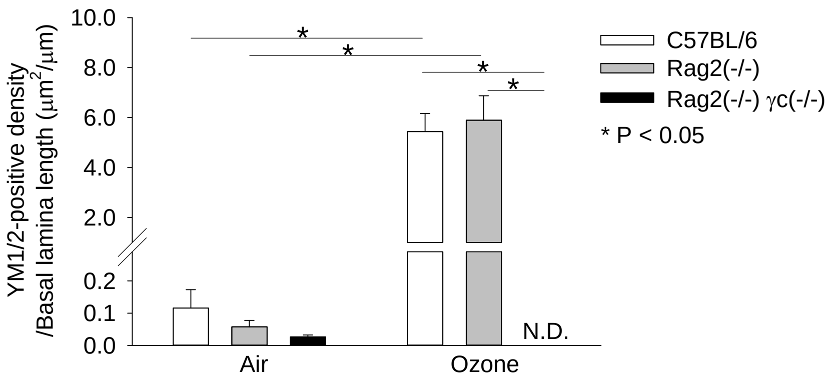
O3 Exposed
T & B Cells Depleted
ILCs Sufficient
Rag2(-/-)

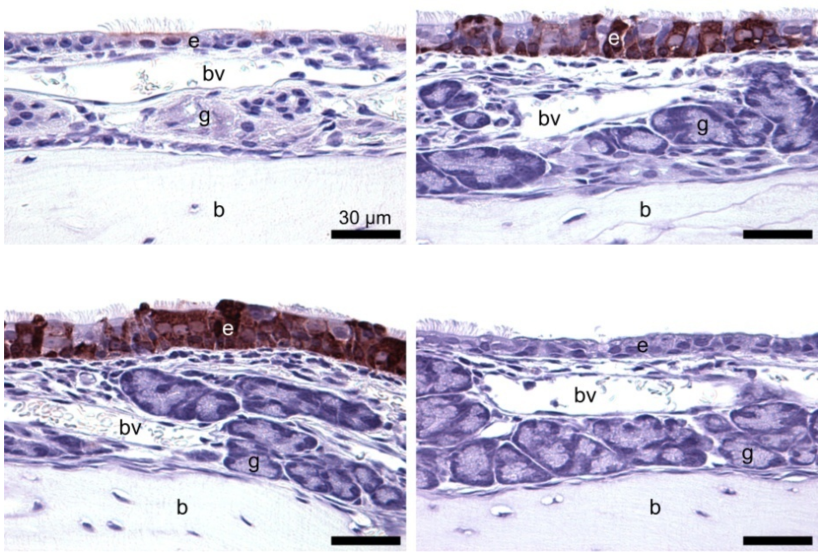
O3 Exposed
T, B, & ILCs Depleted
Rag2(-/-)γc(-/-)

Ozone-Induced Hyalinosis (Ym1/Ym2) is Dependent on Innate Lymphoid Cells

Mouse Strain	Filtered Air	0.8 ppm O3
C57BL/6	6	6
Rag2(-/-)	6	6
Rag2(-/-)ɣc(-/-)	6	6

Volume Density of Epithelial Ym1/Ym2 Protein



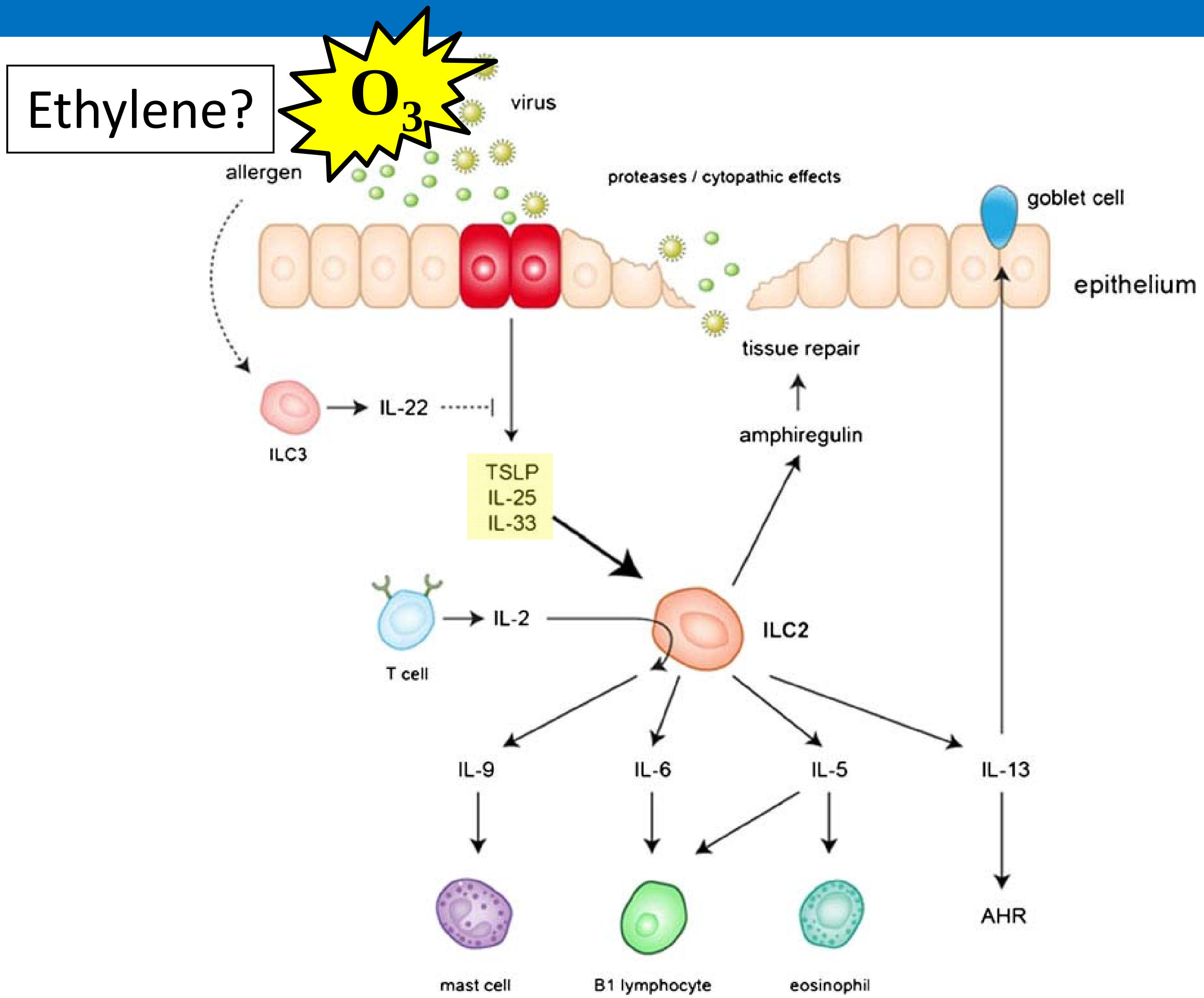


Study Summary and Conclusion

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

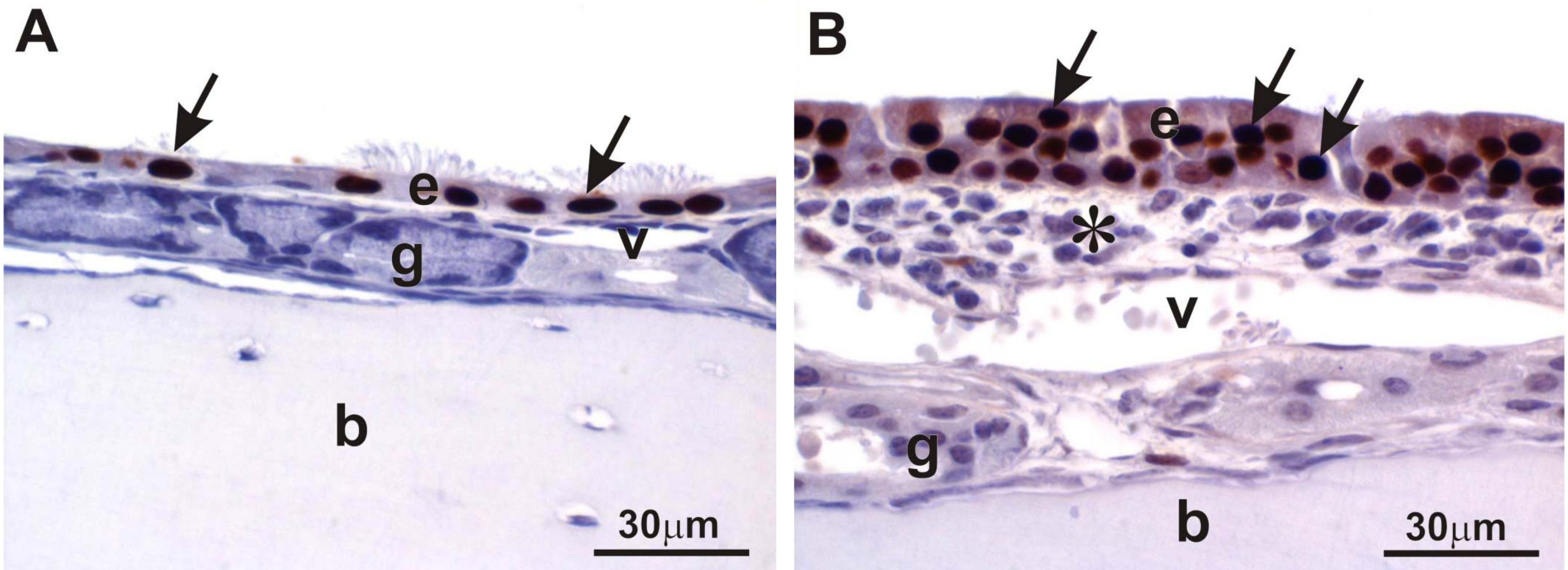
O3-induced eosinophilic rhinitis and nasal epithelial remodeling are mediated by ILCs.

Multifaceted Role of Type 2 Innate Lymphoid Cells (ILC2) in Airway Inflammation



Kein Wolterink RG, Hendriks RW. Curr Allergy Asthma Rep (2013) 13:271-280

IL-33 (alarmin) Immunohistochemistry
C57BL/6 Mice, Nasal Epithelium



Filtered Air Control
0 ppm Ozone

9-day Ozone Exposure
0.5 ppm, 4h/day

Questions to be answered with Current MOA Study

- Will a 12-wk episodic exposure to ethylene followed by a one day ethylene challenge cause nasal lesions that resemble those of a known **respiratory sensitizer** (ortho-phthalaldehyde; OPA) **or a common respiratory irritant** (ozone; O3)?

- **Do the nasal inflammatory and epithelial lesions** caused by inhaled ethylene **persist** after 2 wks postexposure in filtered air?

Questions to be answered with Current MOA Study

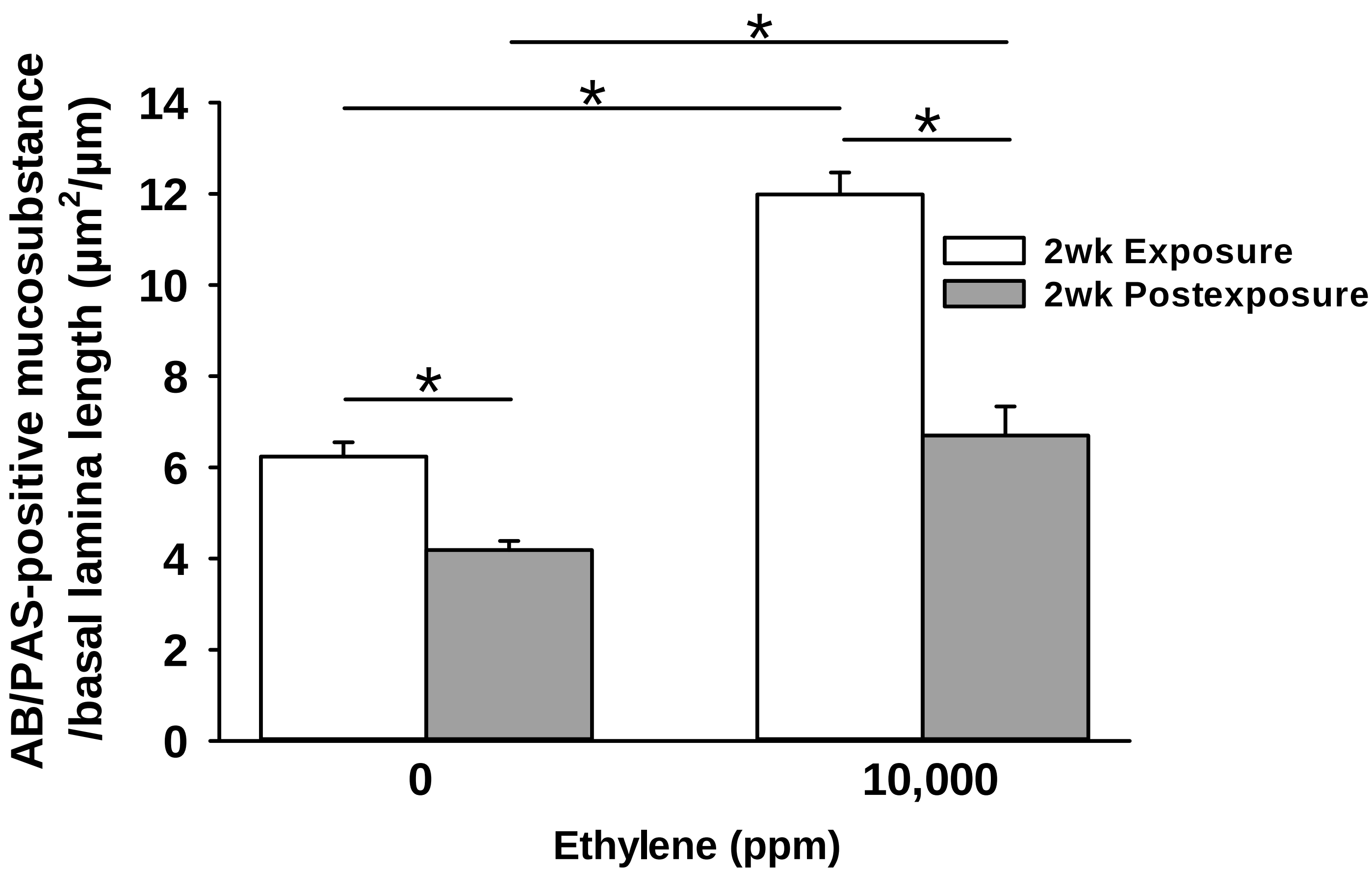
- Will episodic exposures to ethylene cause an **increase in the severity of nasal toxicity with an increase in the number of exposures?**

- **Does the mode of action for the ethylene-induced nasal lesions resemble that of ozone-induced eosinophilic rhinitis** and nasal epithelial remodeling or OPA-induced eosinophilic inflammation?

Current Mode of Action Study

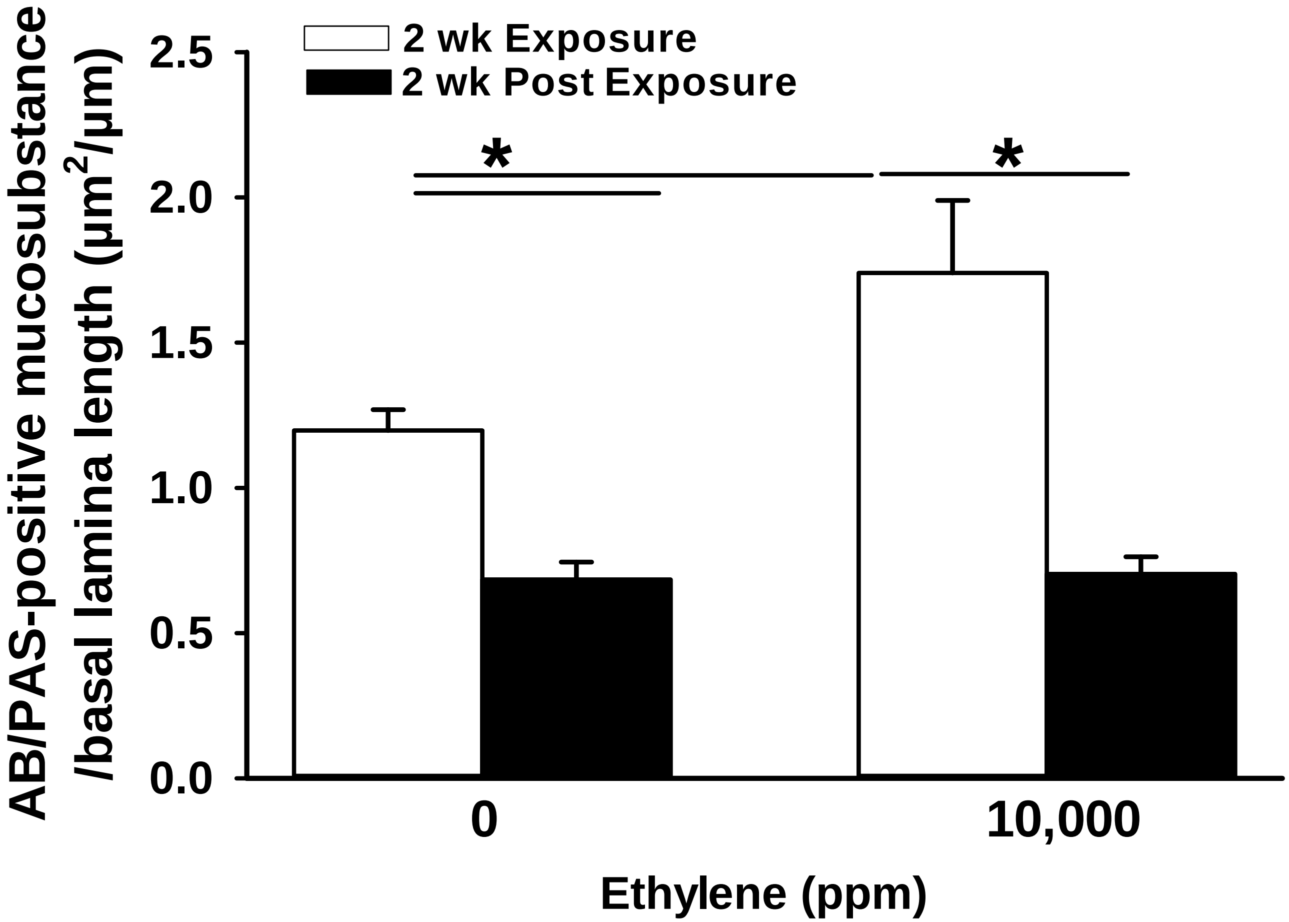
- **Purpose**: To determine if ethylene is a respiratory irritant or sensitizer (Study 1)? Does the mode of action of ethylene resemble that of ozone (Study 2)?
- **Animals**: F344/DuCrl rats (Study 1); C57BL/6 mice, Rag2-/-, and Rag2-/-γc-/- (Study 2)
- **Episodic exposure**: 2wk ethylene (5d/wk; 10,000 ppm)→ 2wk filtered air (0 ppm)→ 2wk ethylene→ 2wk filtered air→ 2wk ethylene→ 2wk filtered air→ 1-day ethylene challenge
- **Endpoints**: Nasal histopathology; morphometry; RT-PCR (mRNA expression of inflammatory cytokines and epithelial proteins (e.g., secretory); pulmonary function (rat)

Initial Results: Epithelial Mucosubstances in Rat Nasopharyngeal Meatus (2wk-Ethylene Exposure and 2wk Postexposure)



* Significantly different, $p \leq 0.05$

Initial Results: Epithelial Mucosubstances in Mouse Nasopharyngeal Meatus (2wk-Ethylene Exposure and 2wk Postexposure)



* Significantly different, $p \leq 0.05$

Summary

✓ Comparative nasal anatomy

✓ Nasal toxicity of ethylene and ozone

✓ Mode of action of ozone-induced nasal pathology

✓ Inhalation study to understand the mode of action of ethylene-induced nasal pathology

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Questions?

Thank you!

Caricature by David Levine, New York Review of Books