

Airborne Particle Effects on the Respiratory System of Sensitive Animals and Asthmatic Humans

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Rationale

- Studies suggest an association between ambient particulate matter and increased morbidity in individuals with allergic airway disease
- Brown Norway rats can serve as a model of allergic airway disease due a strong Th2 response following sensitization and challenge with OVA

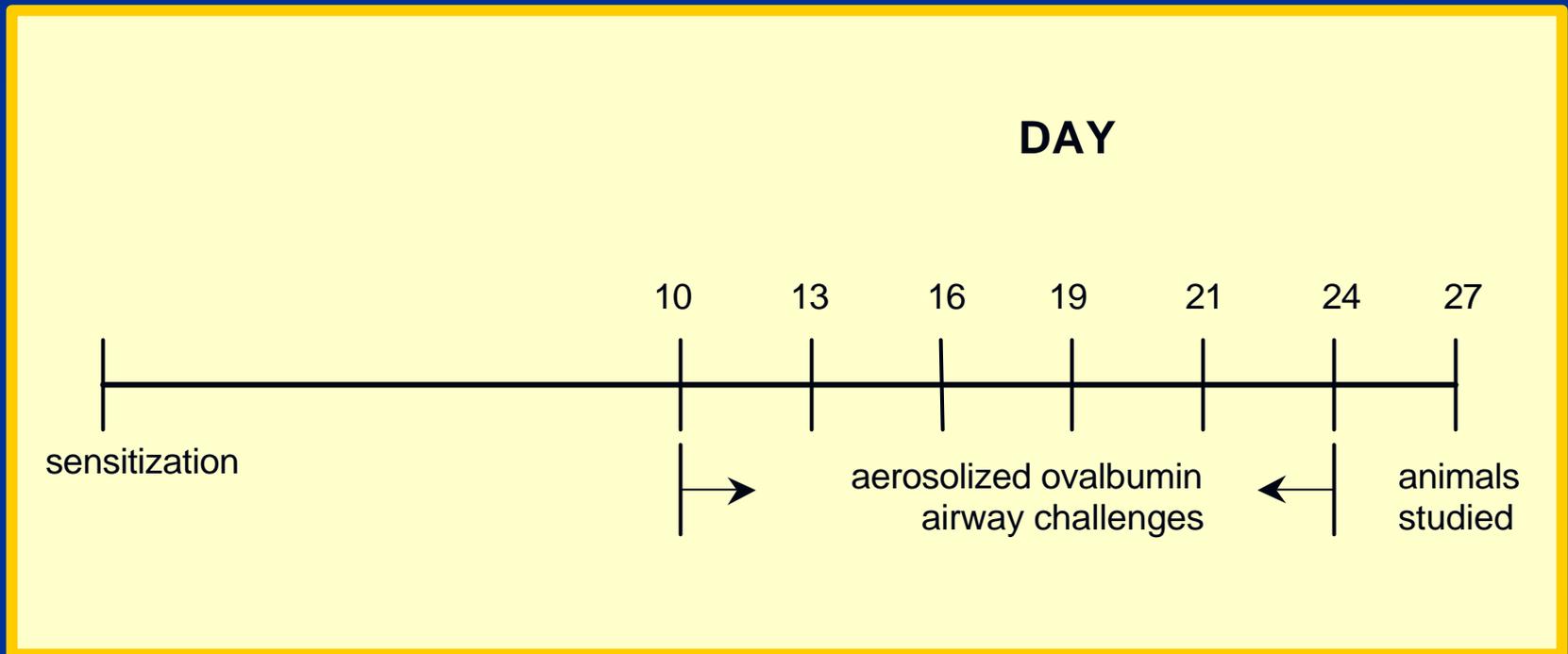
Study Objective One

- To establish a model of airway reactivity in the Brown Norway rat through a process of sensitization and repeated challenge with ovalbumin.

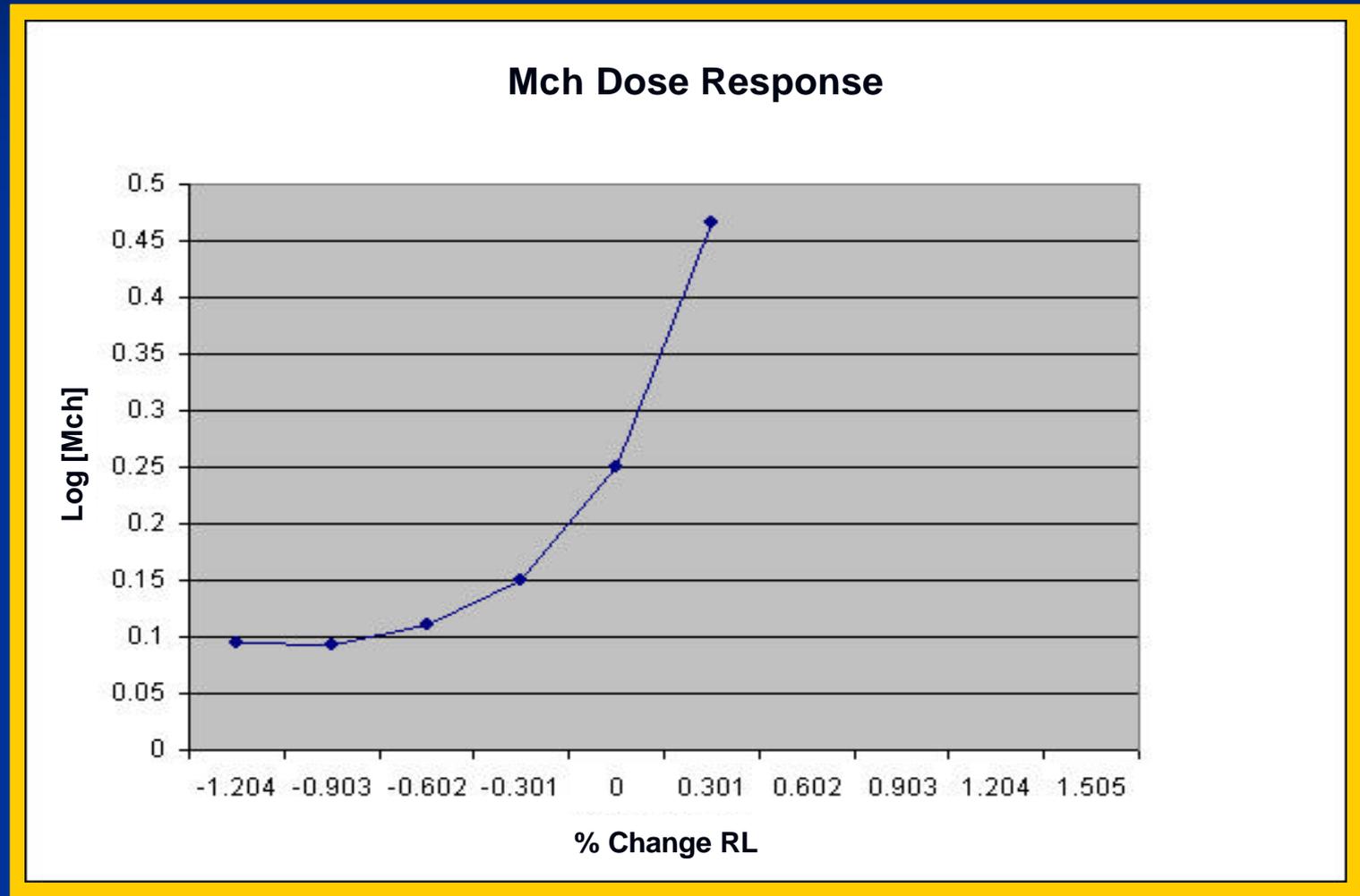
Approach

- Characterize airway reactivity in BN rats sensitized and challenged with OVA
- Quantify intraepithelial cell mucosubstance content of the airways
- Measure changes in eosinophils and mast cells in the lungs
- Measure the degree of inflammation present in the lungs (centriacinar regions)

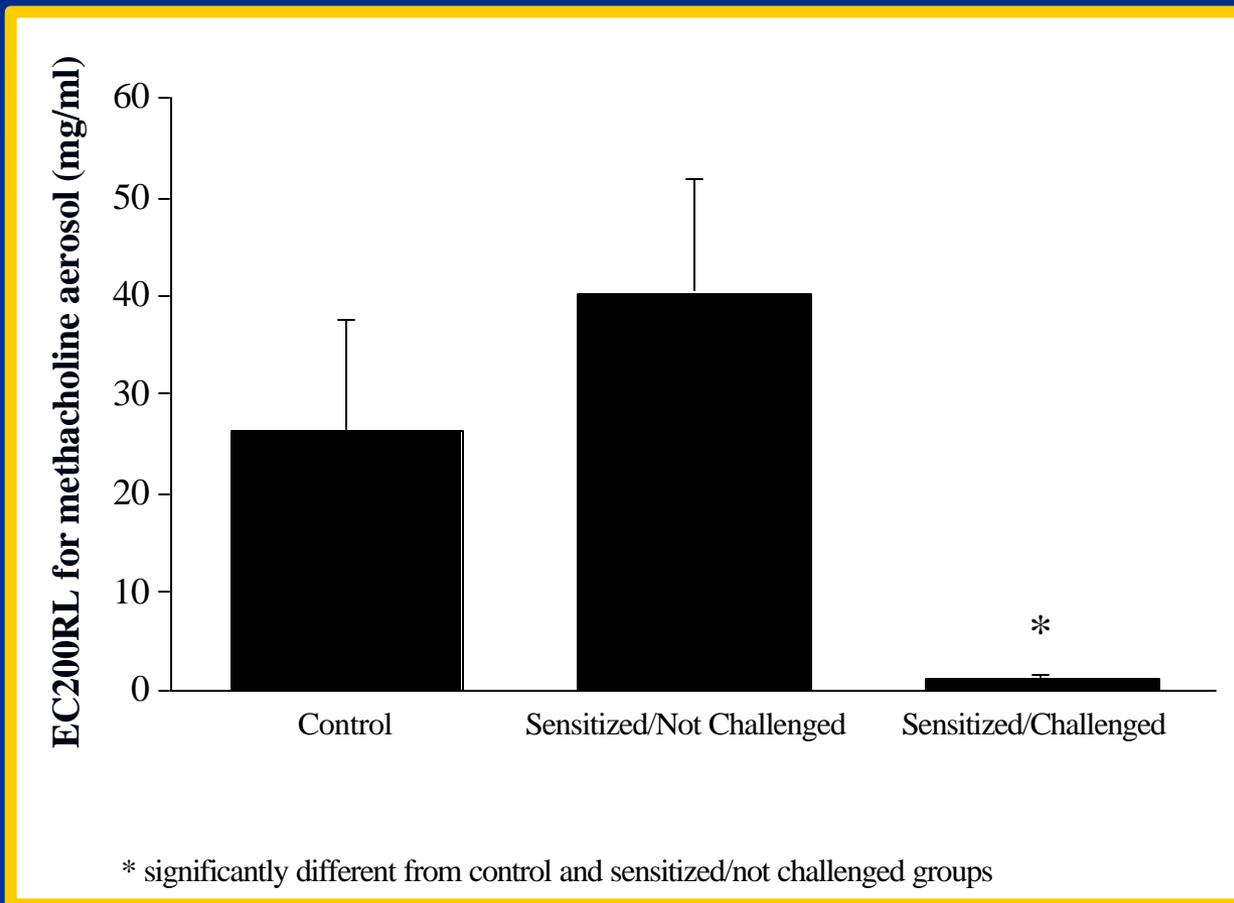
Activity Time-line



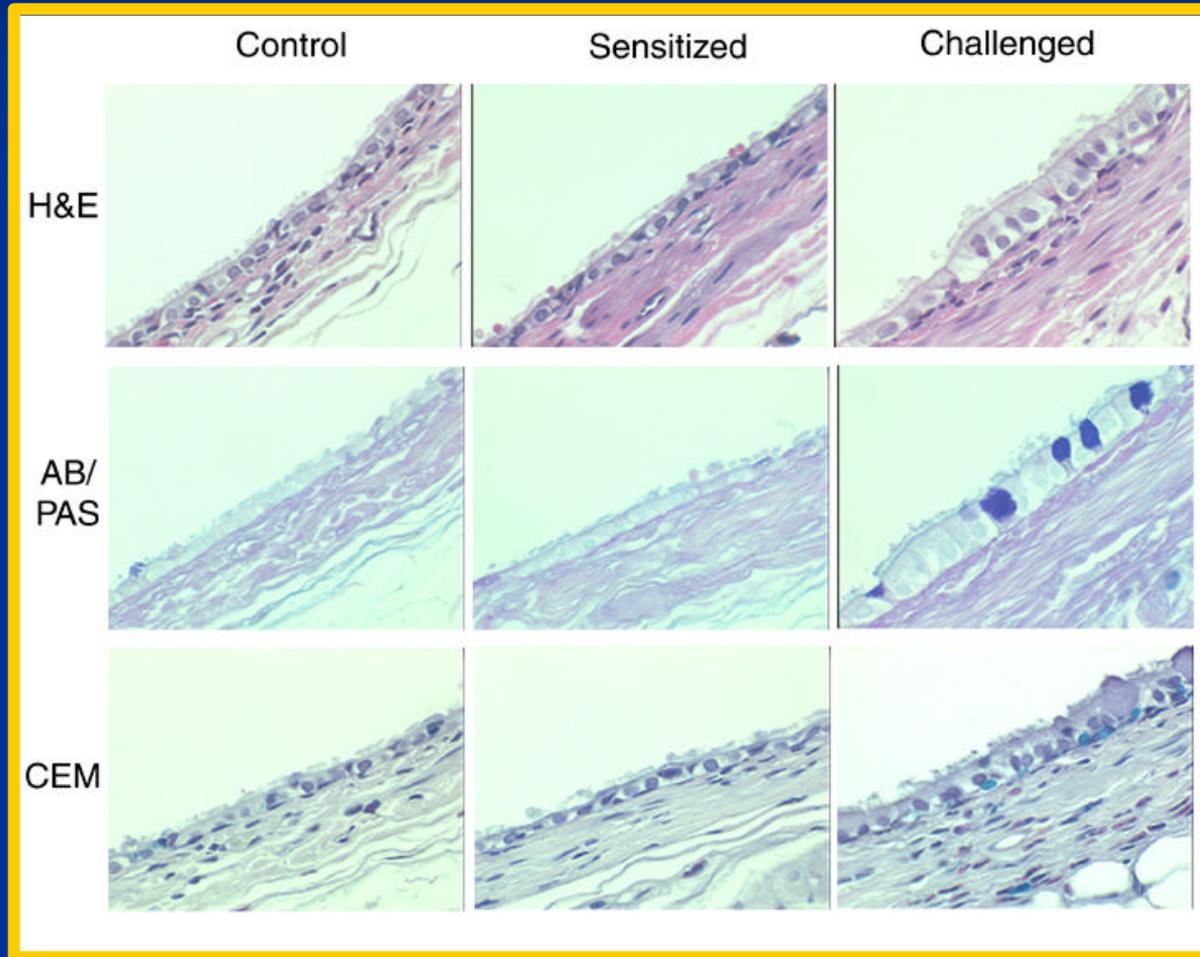
EC200RL Assay: the effective concentration of methacholine required to double lung resistance



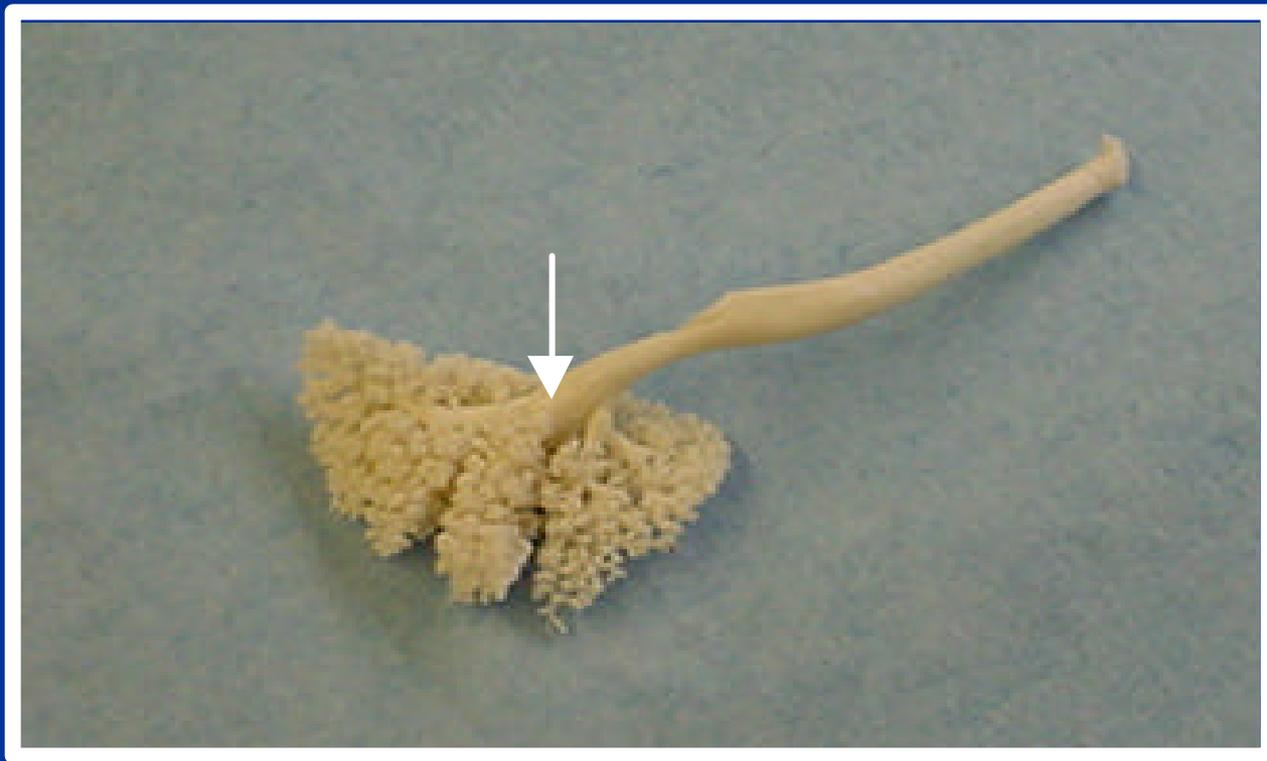
Effects of Ovalbumin Sensitization and Aerosol Challenge on Airway Hyperresponsiveness in Brown Norway Rats



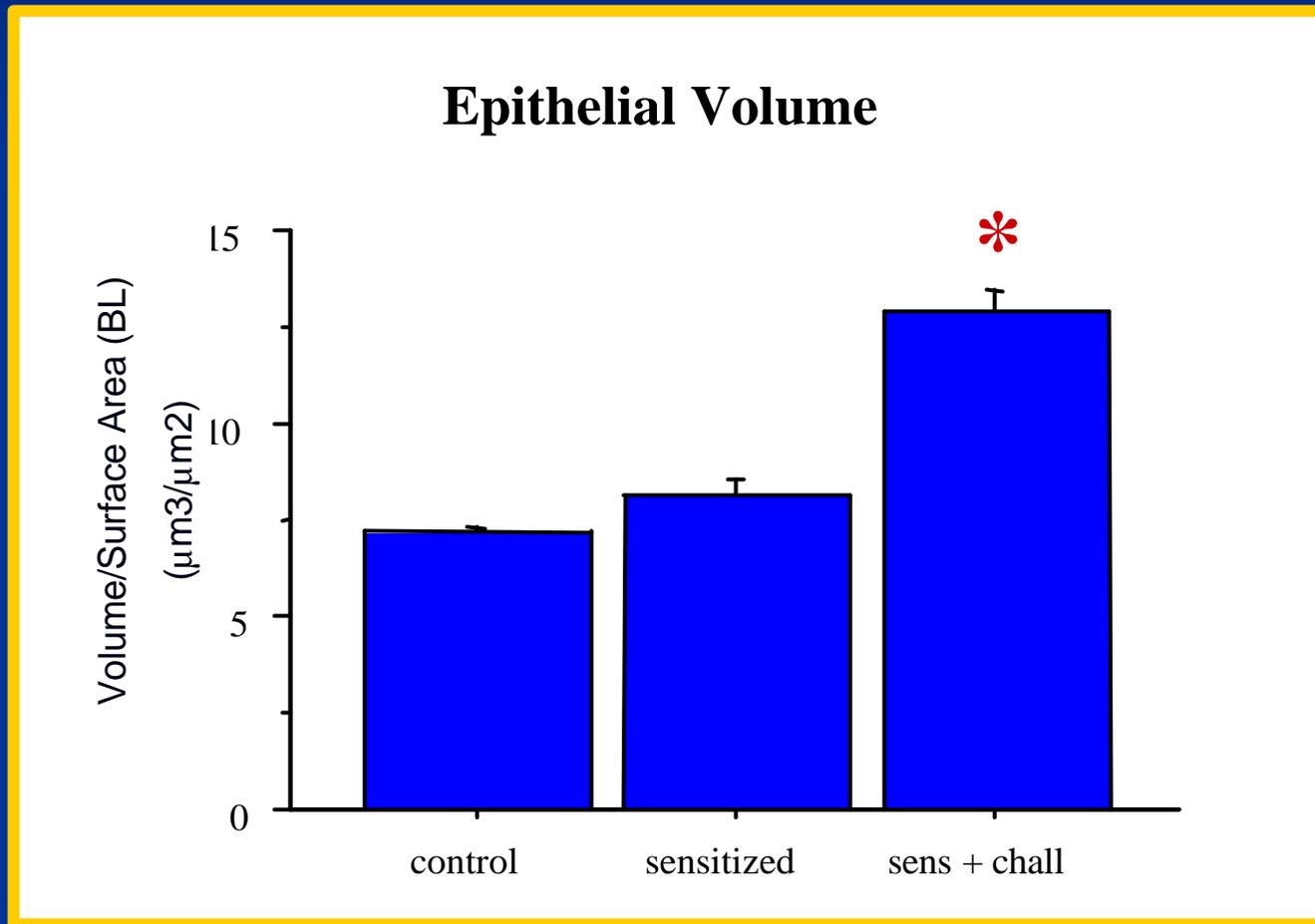
Histochemical Staining of Central Airway



Left Lung Corrosion Cast



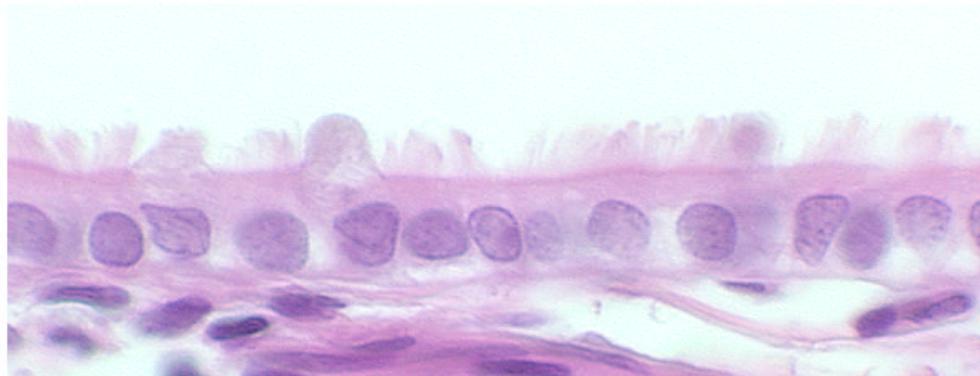
Epithelial Cell Volume of the Central Airway



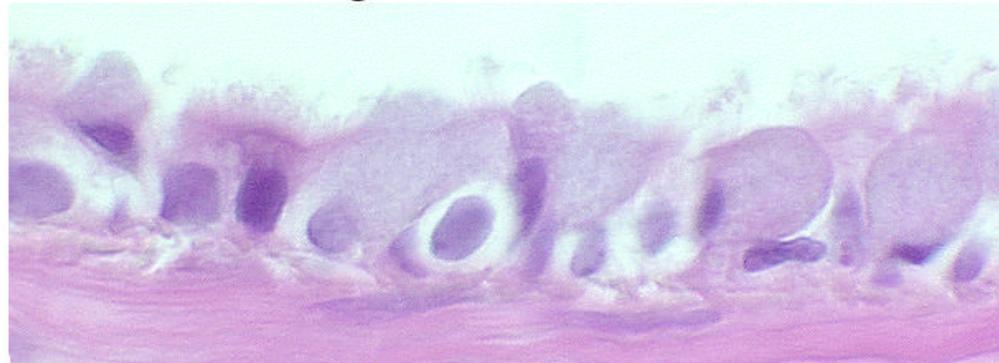
Alcian Blue/Periodic Acid Schiff Staining of Central Airway Epithelium

EPITHELIUM OF PROXIMAL AIRWAY

Control Animal H&E Stain

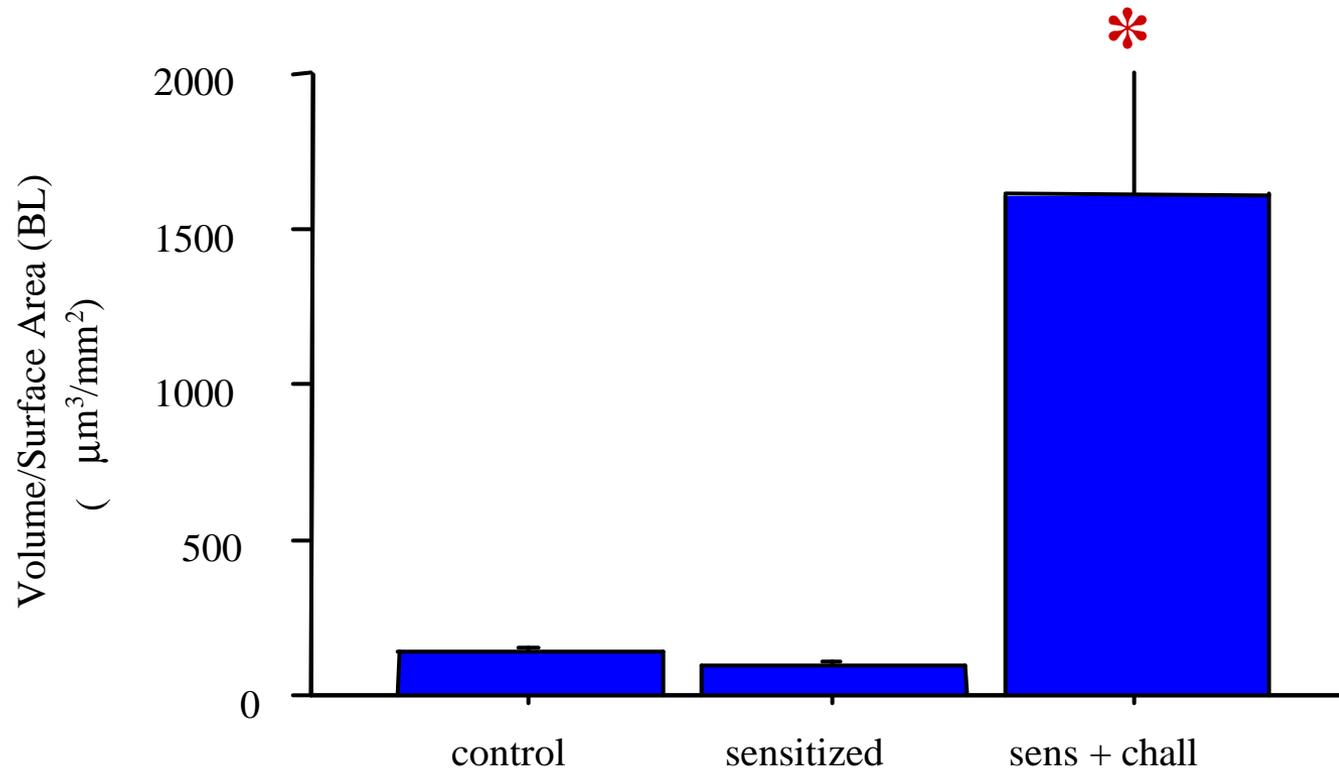


Challenged Animal H&E Stain

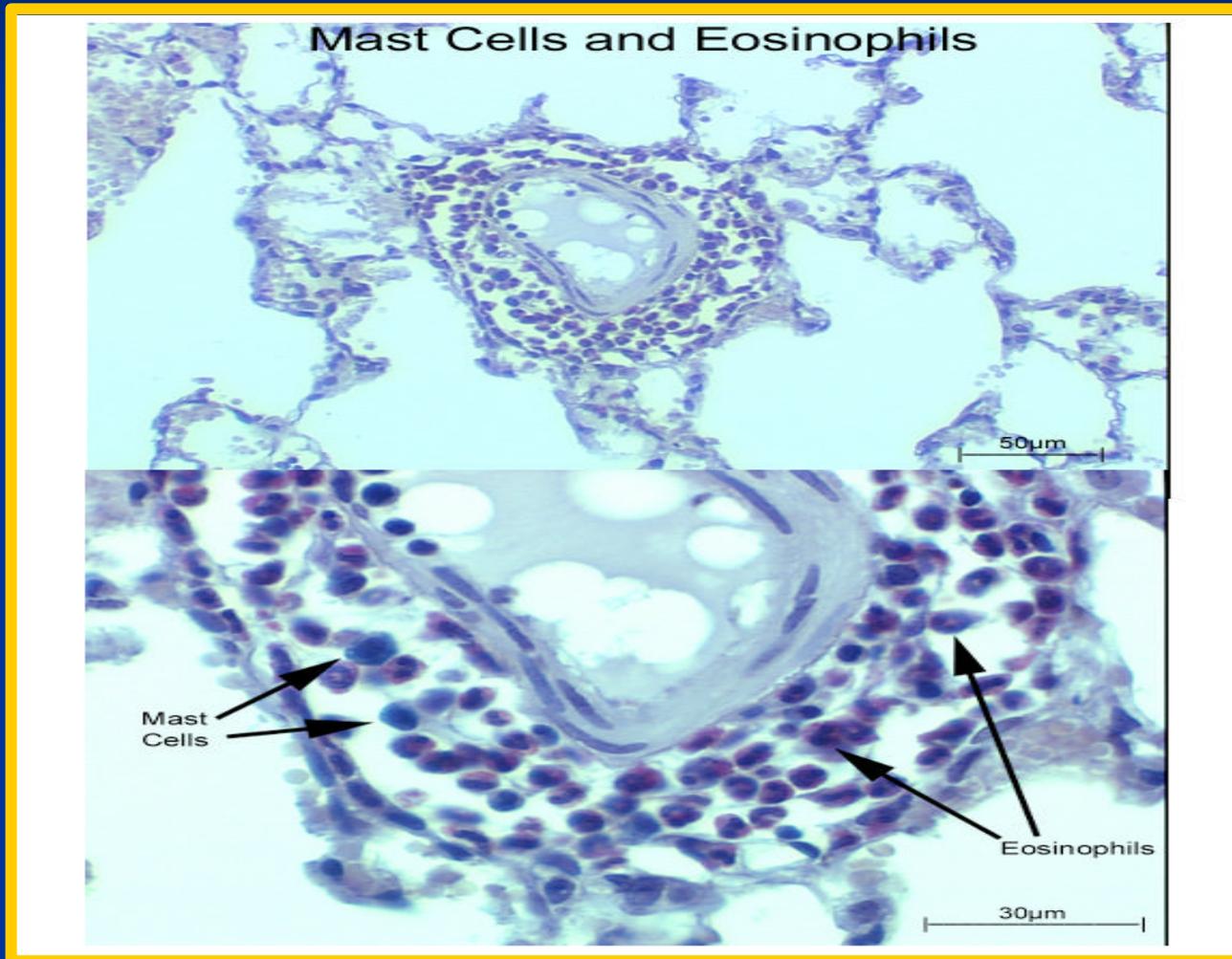


Volume of Intracellular Mucosubstances of the Central Airway

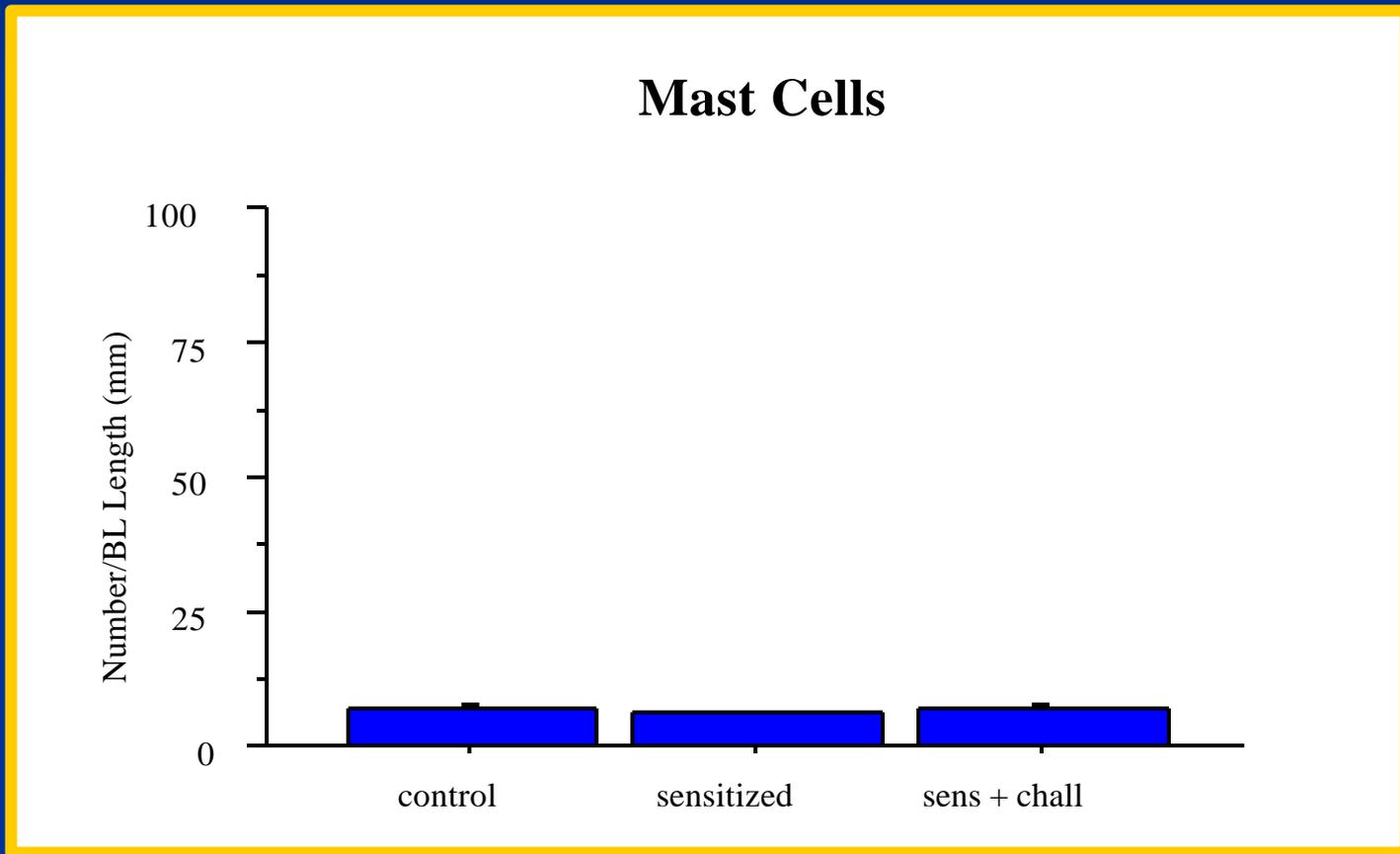
Mucosubstance Volume



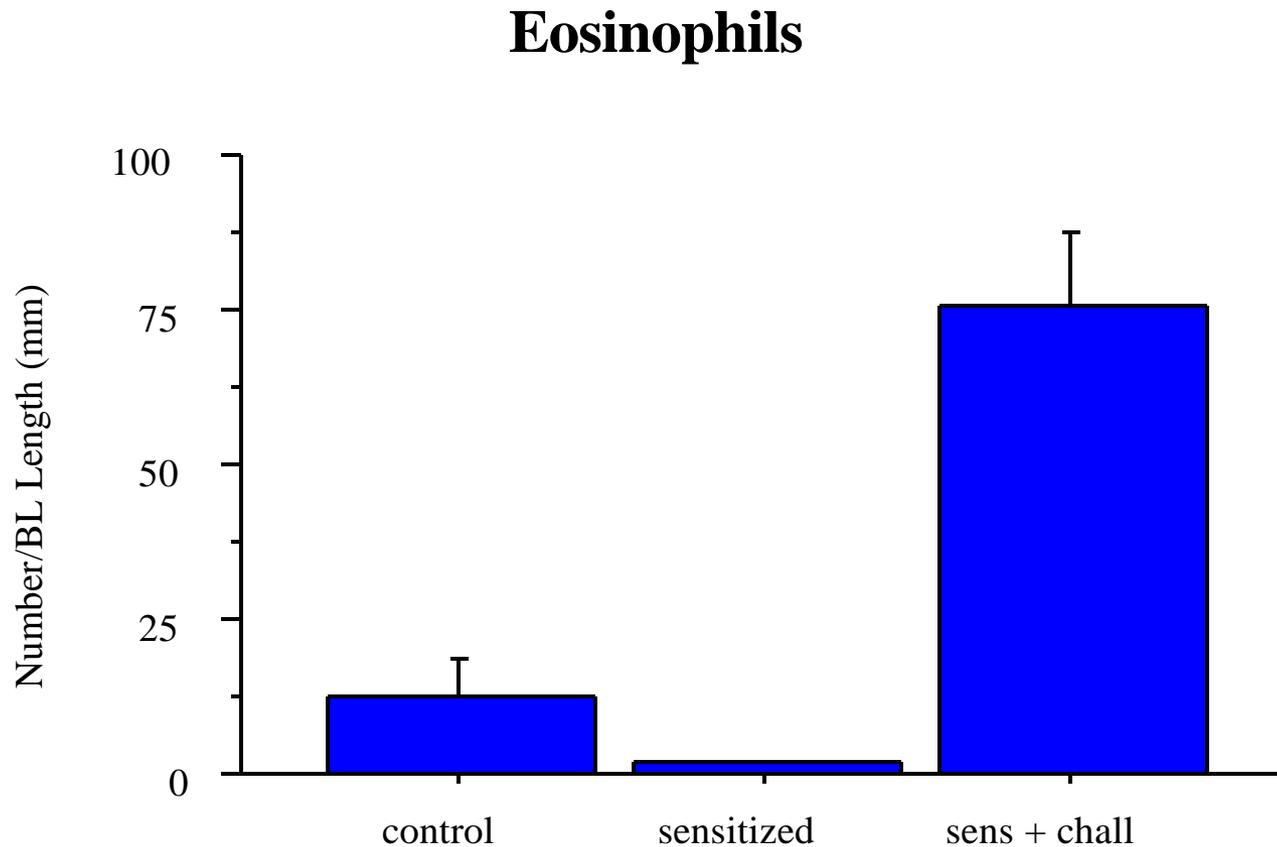
Perivascular (PV) Mast Cells and Eosinophils



Number of PV Mast Cells



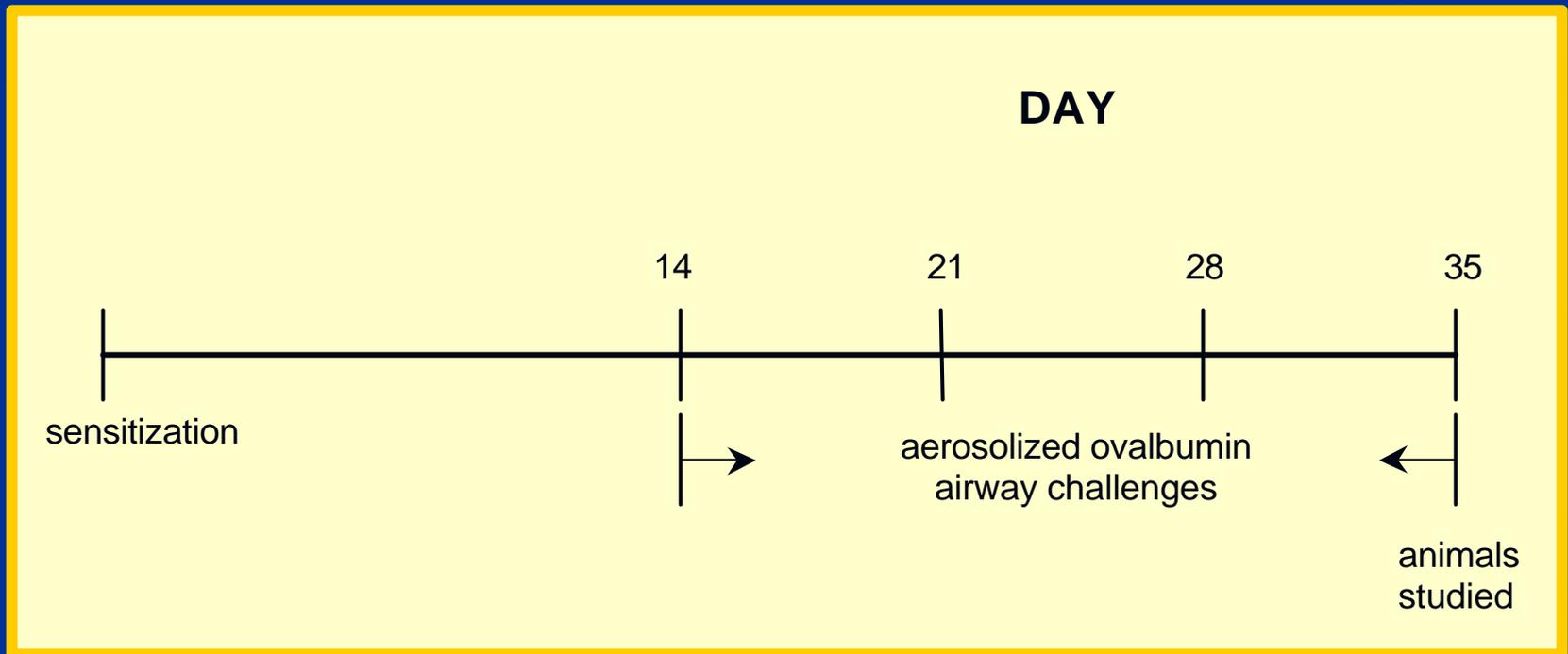
Number of PV Eosinophils



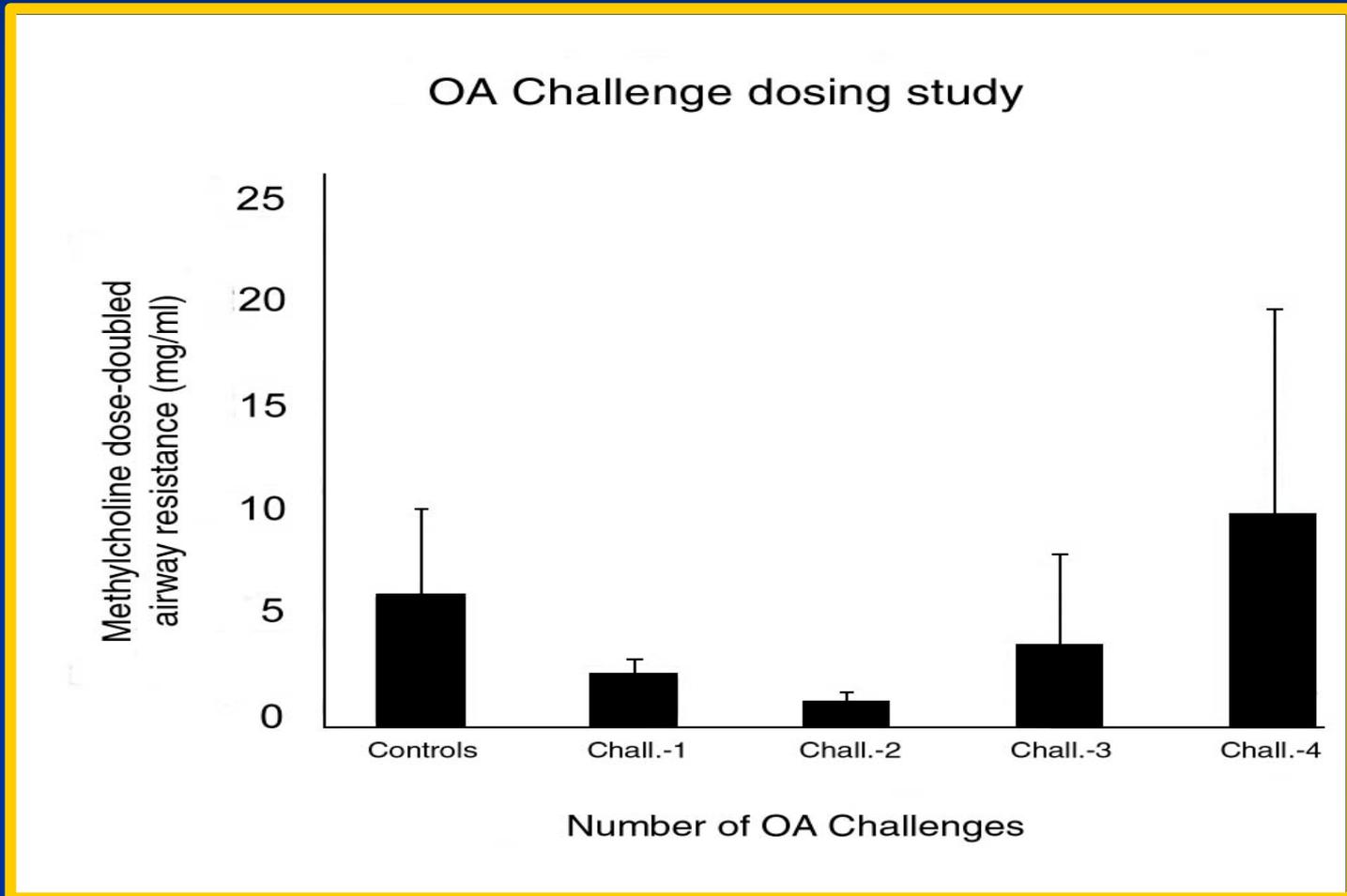
Study Objective Two

- To determine the most optimal conditions for a model of allergic airways in the Brown Norway rat by repeated weekly challenges with ovalbumin.

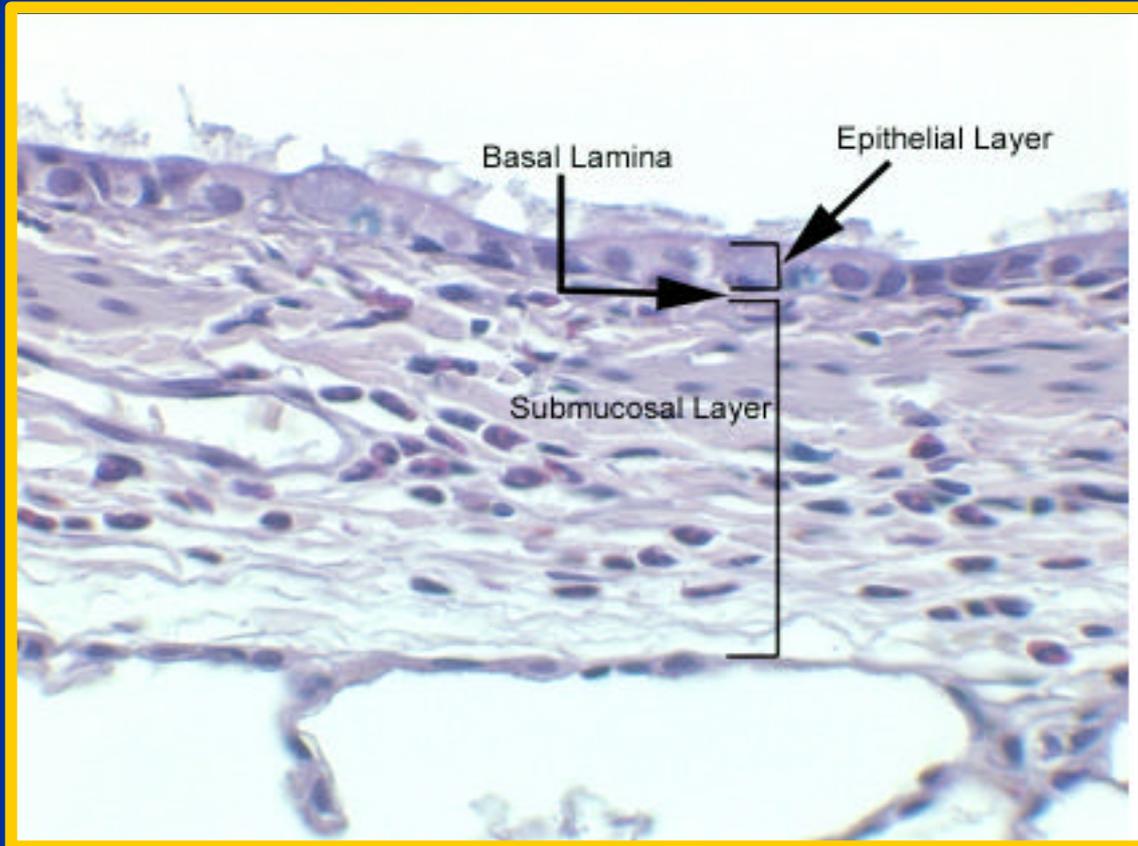
Activity Time-line



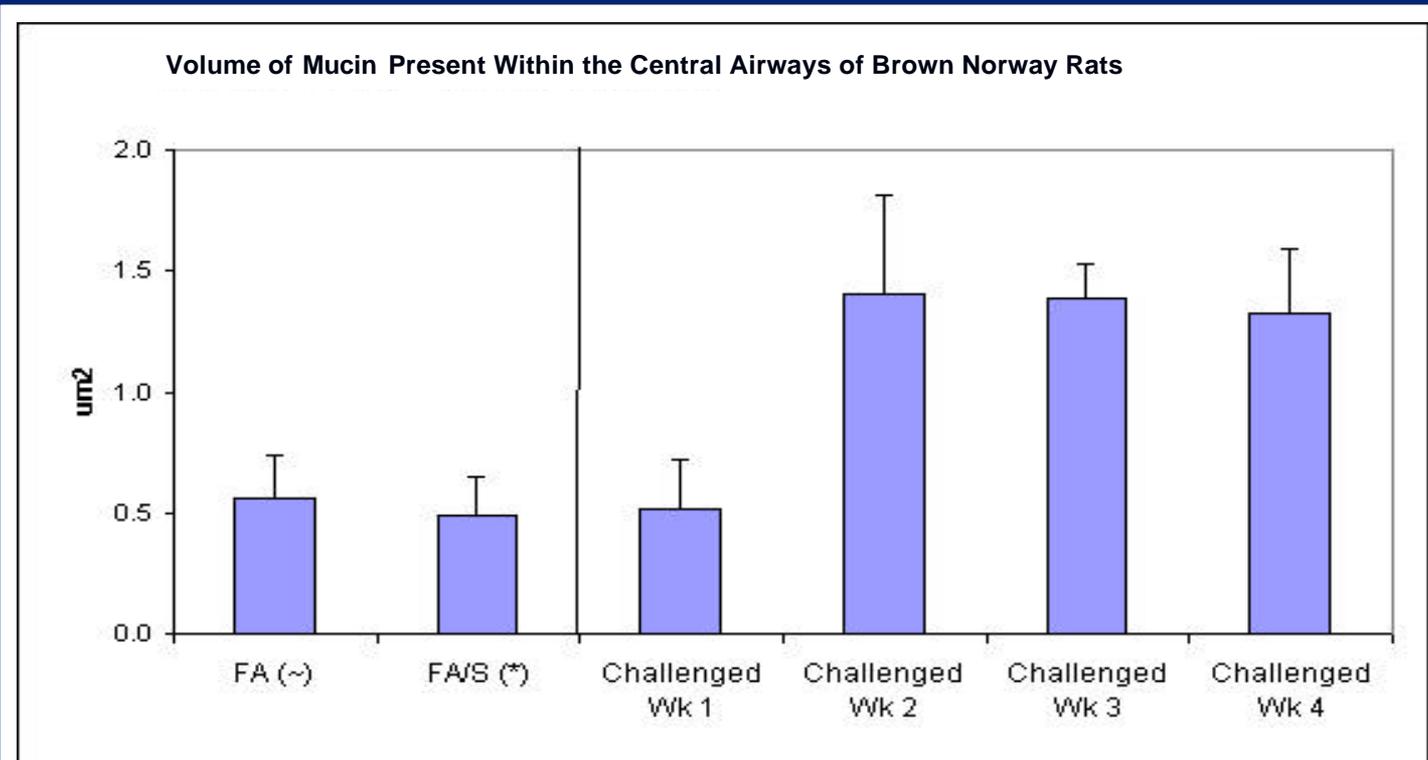
EC200RL: The effective dose required to double lung resistance



Central Airway Wall Composition

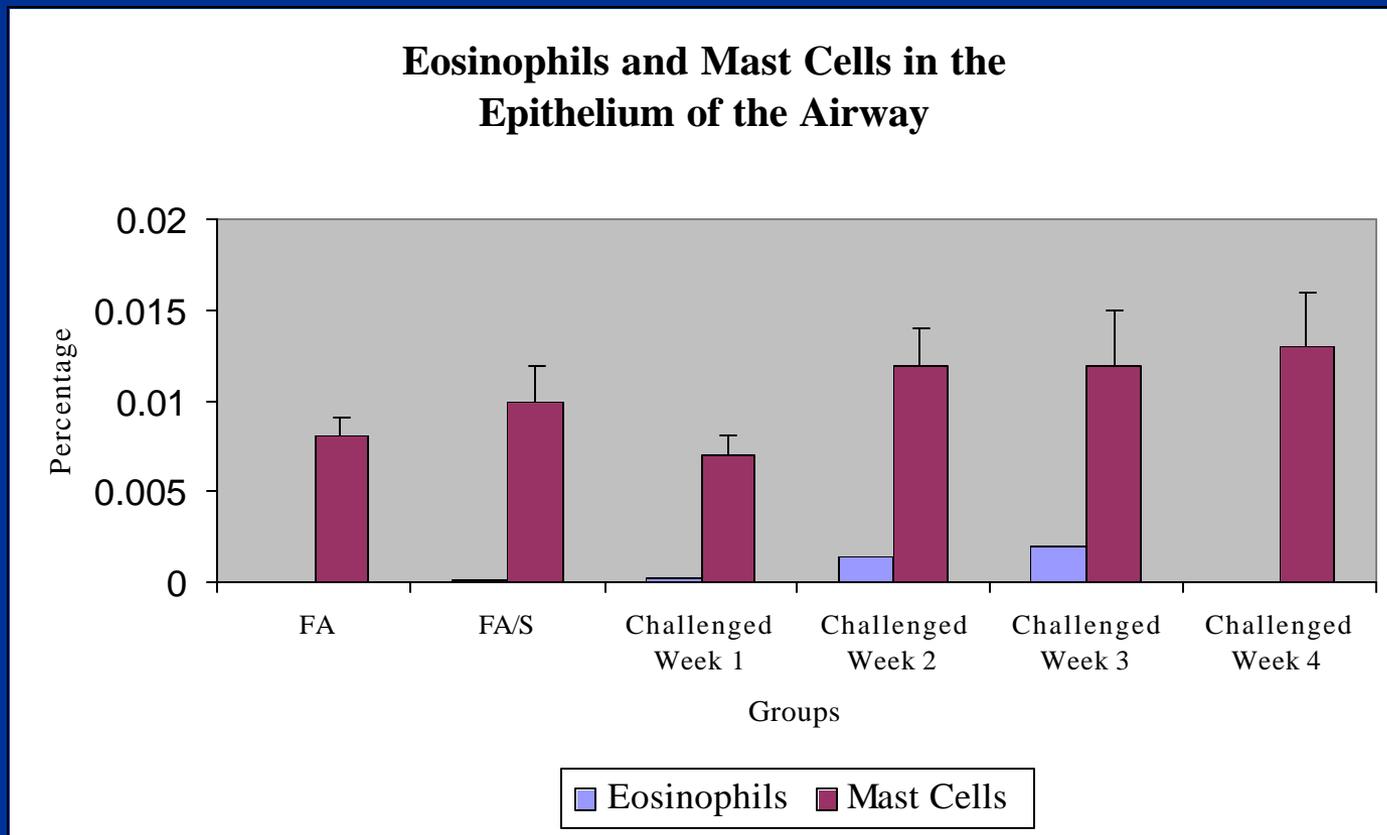


Volume of Mucin Present Within the Central Airways of Brown Norway Rats



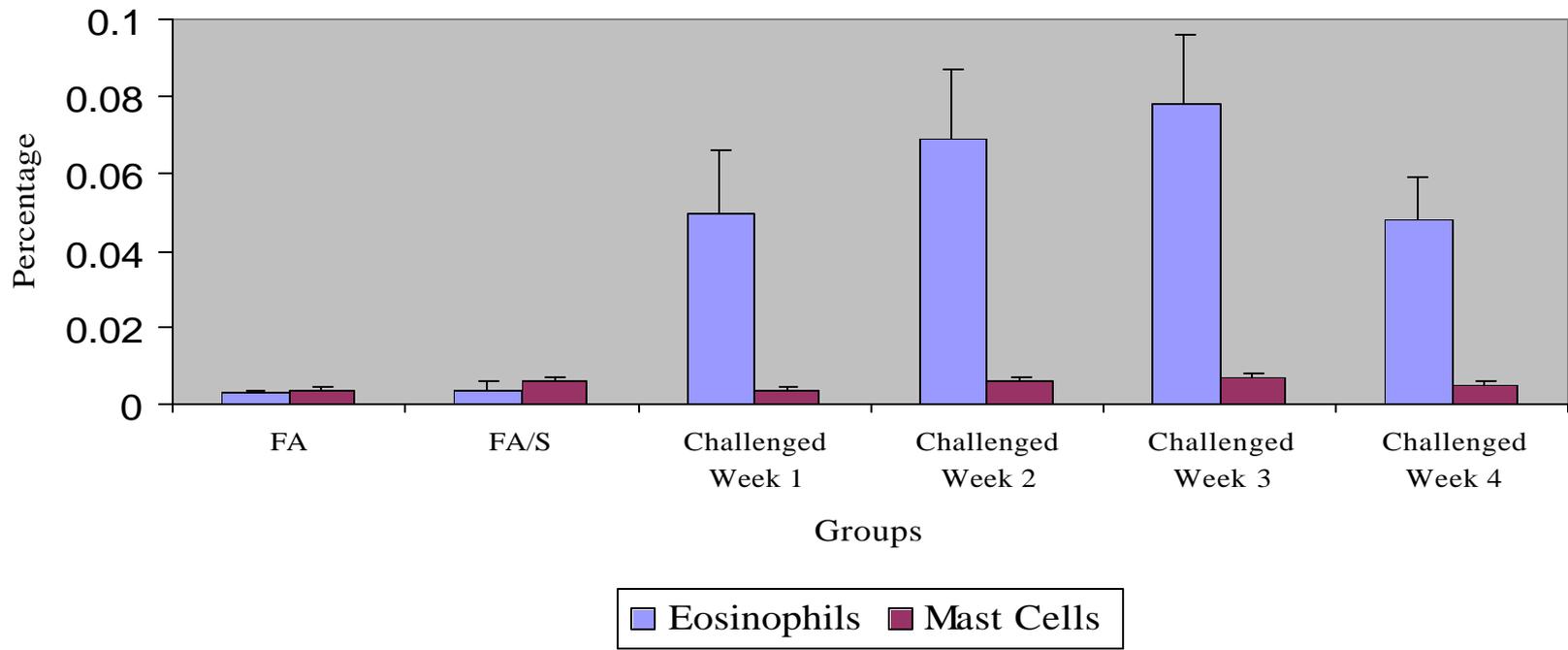
Group	FA (~)	FA/S (*)	Challenged Wk 1	Challenged Wk 2	Challenged Wk 3	Challenged Wk 4
um2 mucin	0.563	0.485	0.514	1.405	1.388	1.323
std err	0.176	0.162	0.203	0.41	0.145	0.268
n	4	8	4	4	4	4

Effects of OVA on Eosinophils and Mast Cells

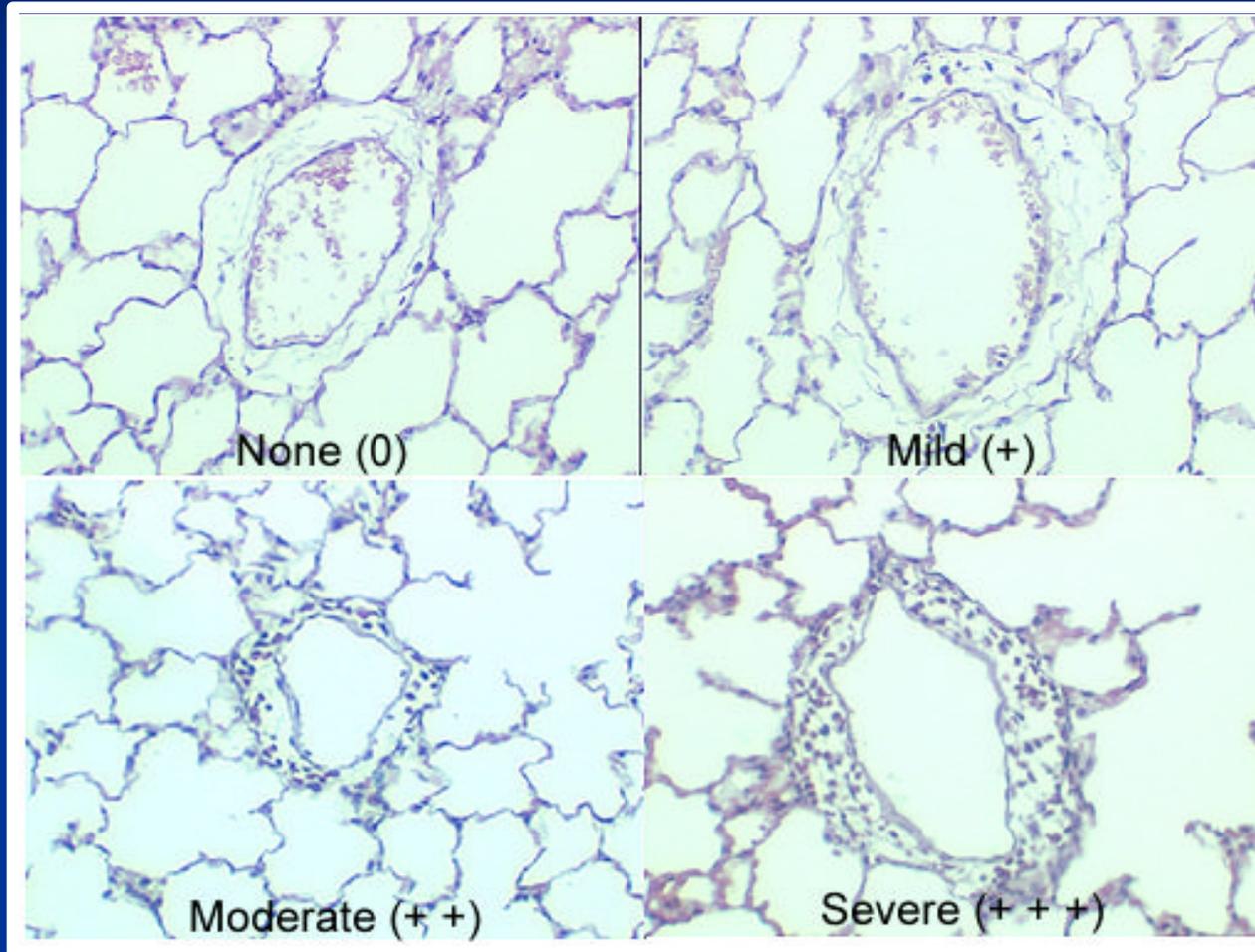


Eosinophil Increases Following OVA Challenges

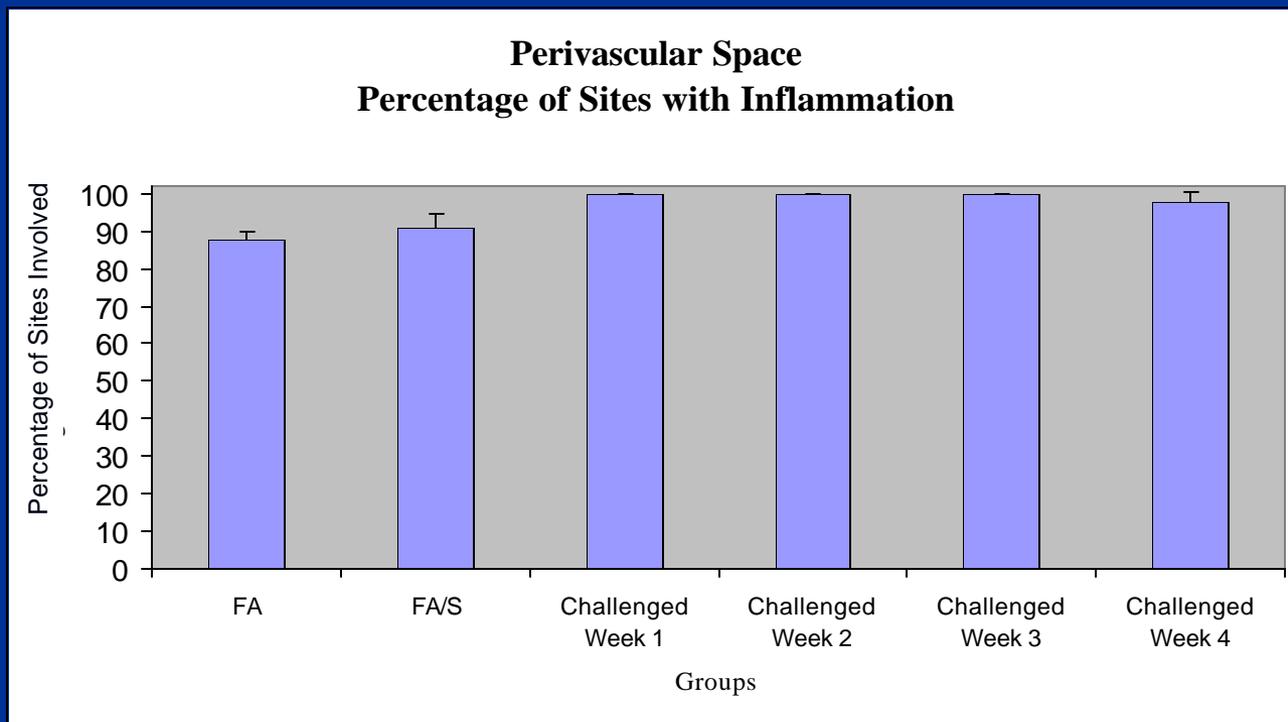
Eosinophils and Mast Cells in the Submucosa of the Airway



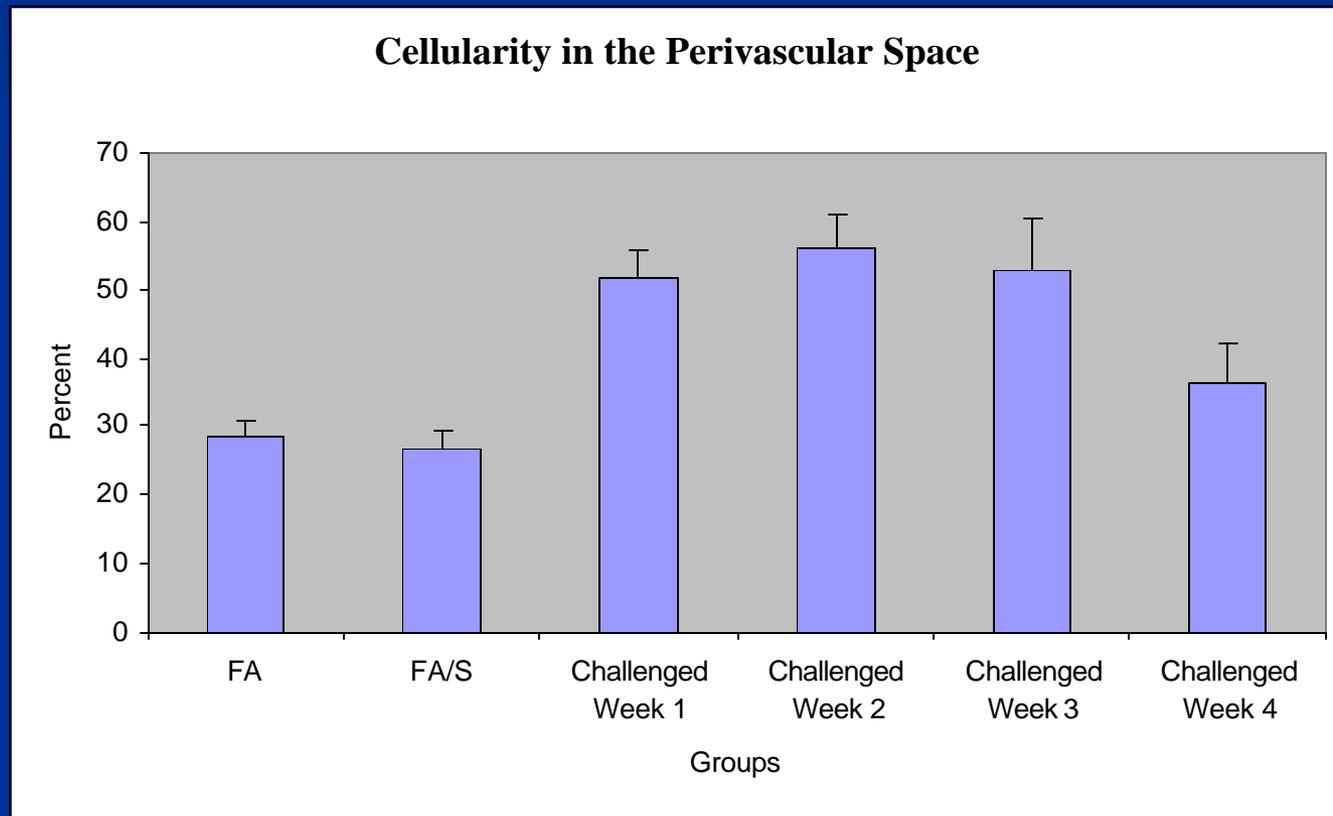
Blood Vessel Scoring of Perivascular Cell Influx



High Levels of Inflammation in Perivascular Space

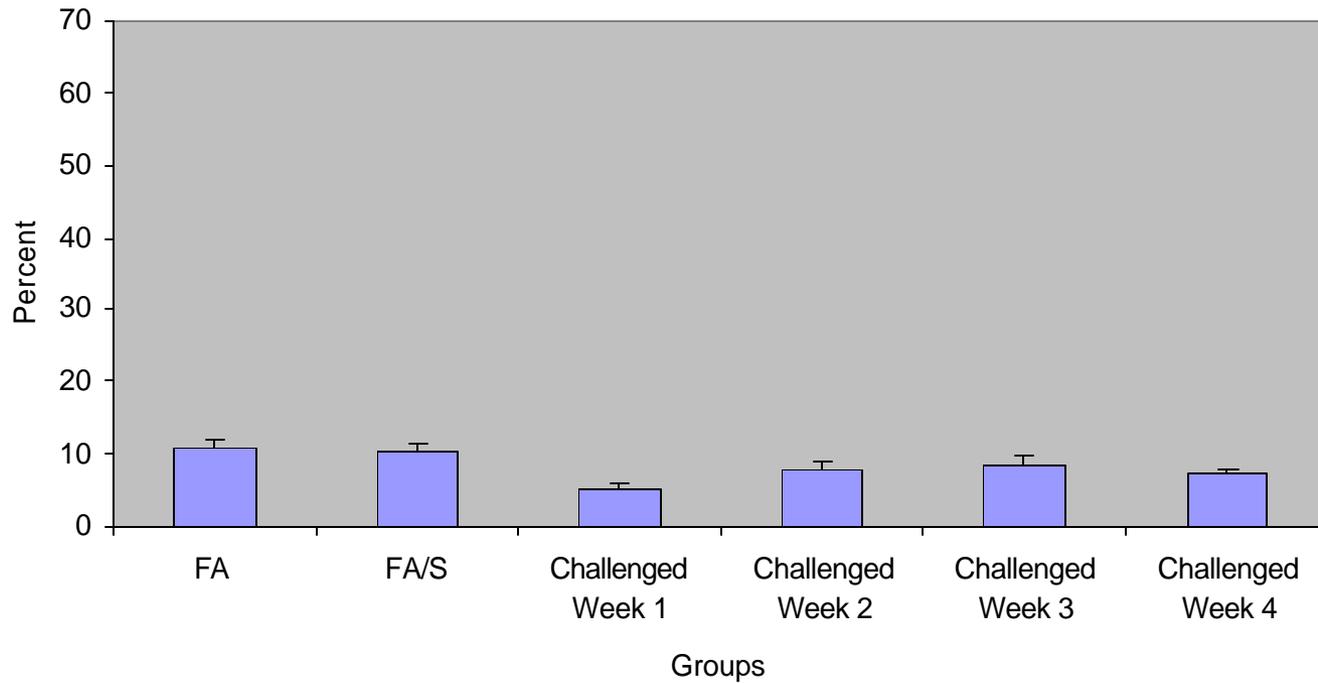


Cellularity

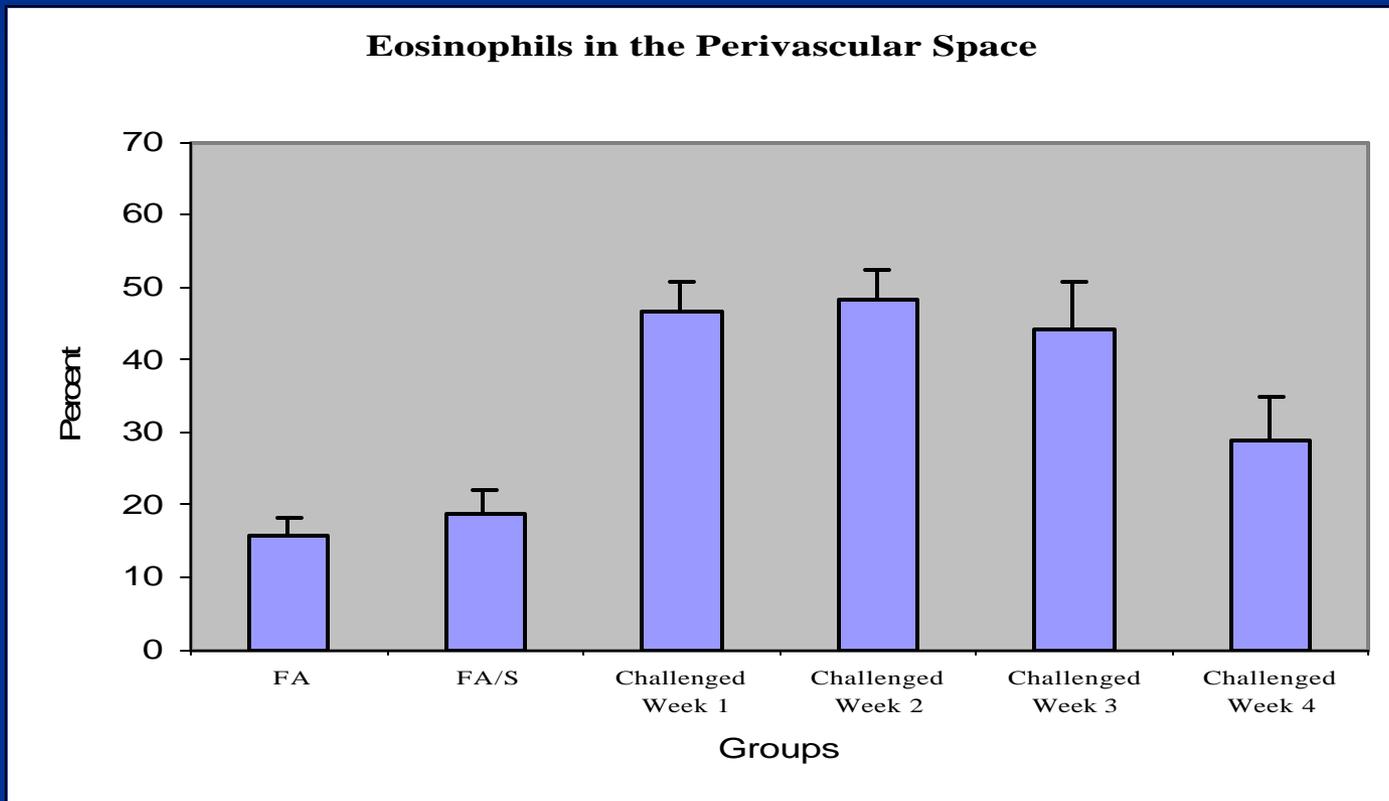


Mast Cells

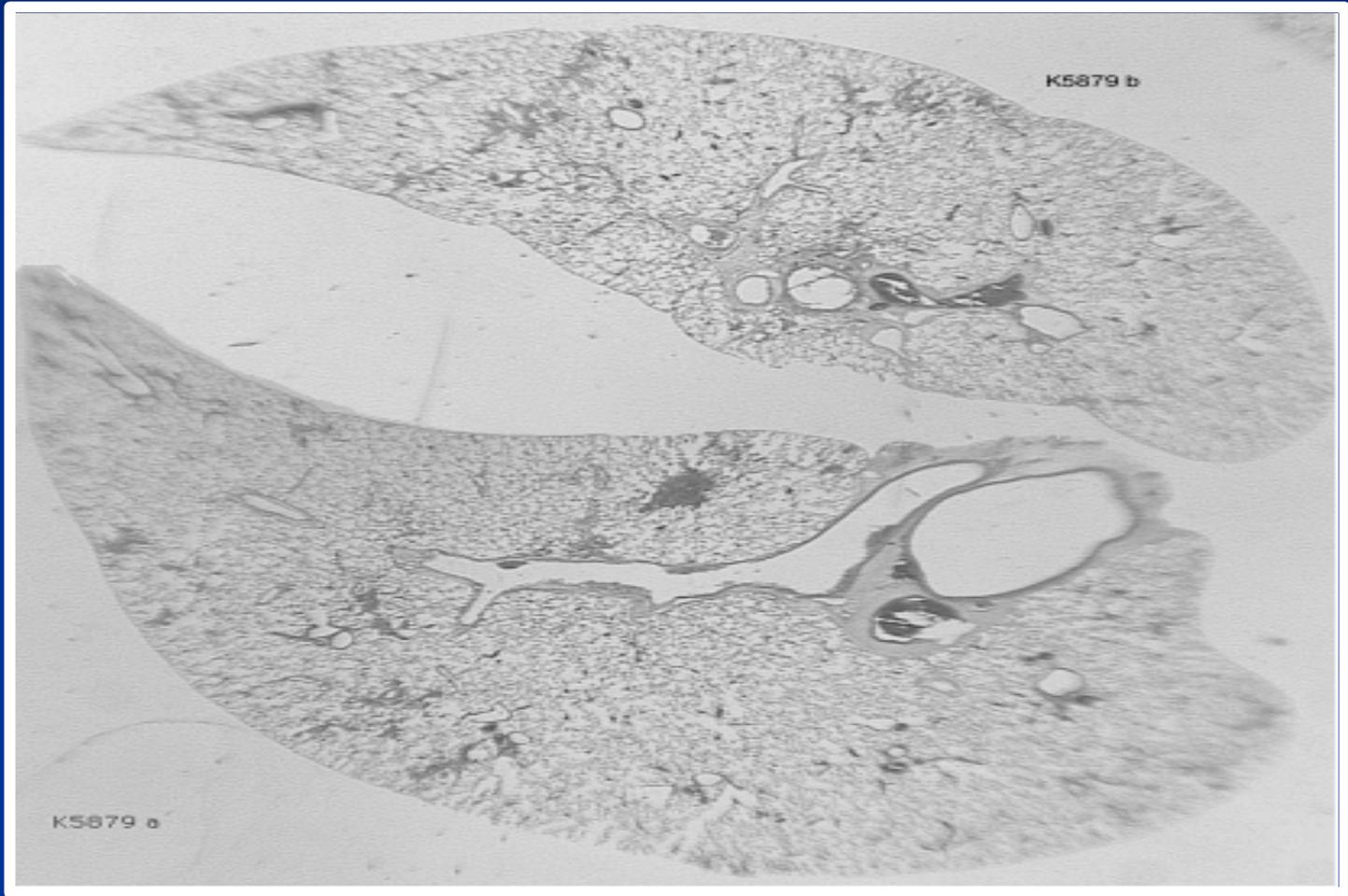
Mast Cells in the Perivascular Space



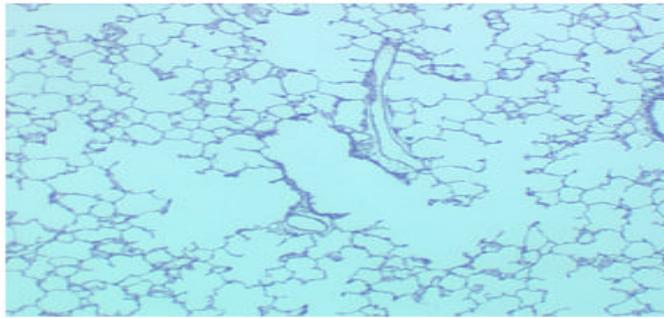
Eosinophils



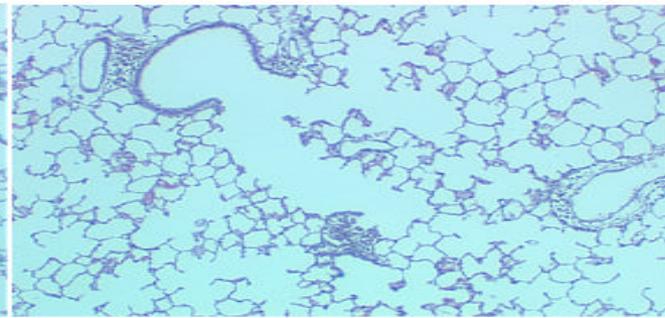
Transverse Lung Tissue Sections



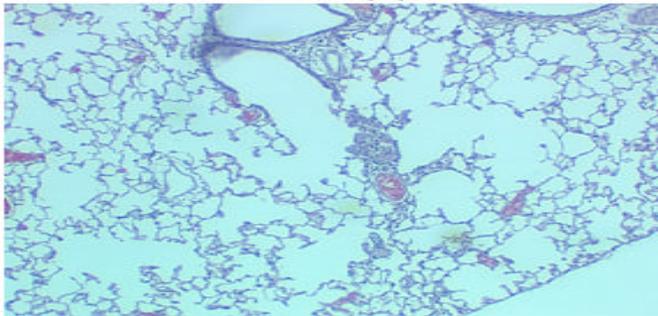
Centriacinar Region (BADJ) Scoring



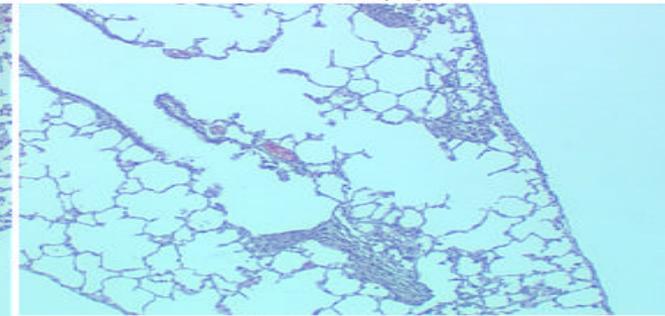
None (0)



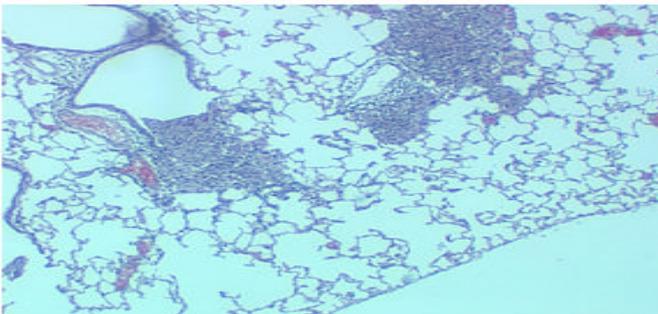
Mild (+)



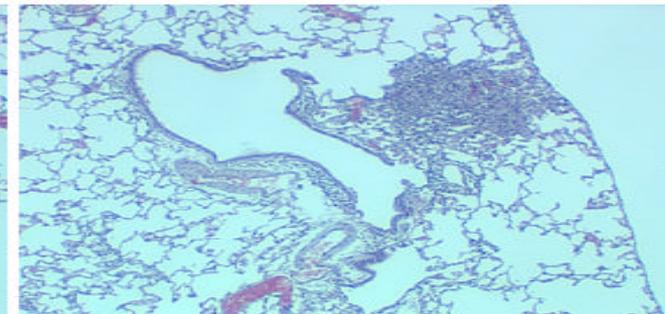
Moderate (++)



Moderate (++)

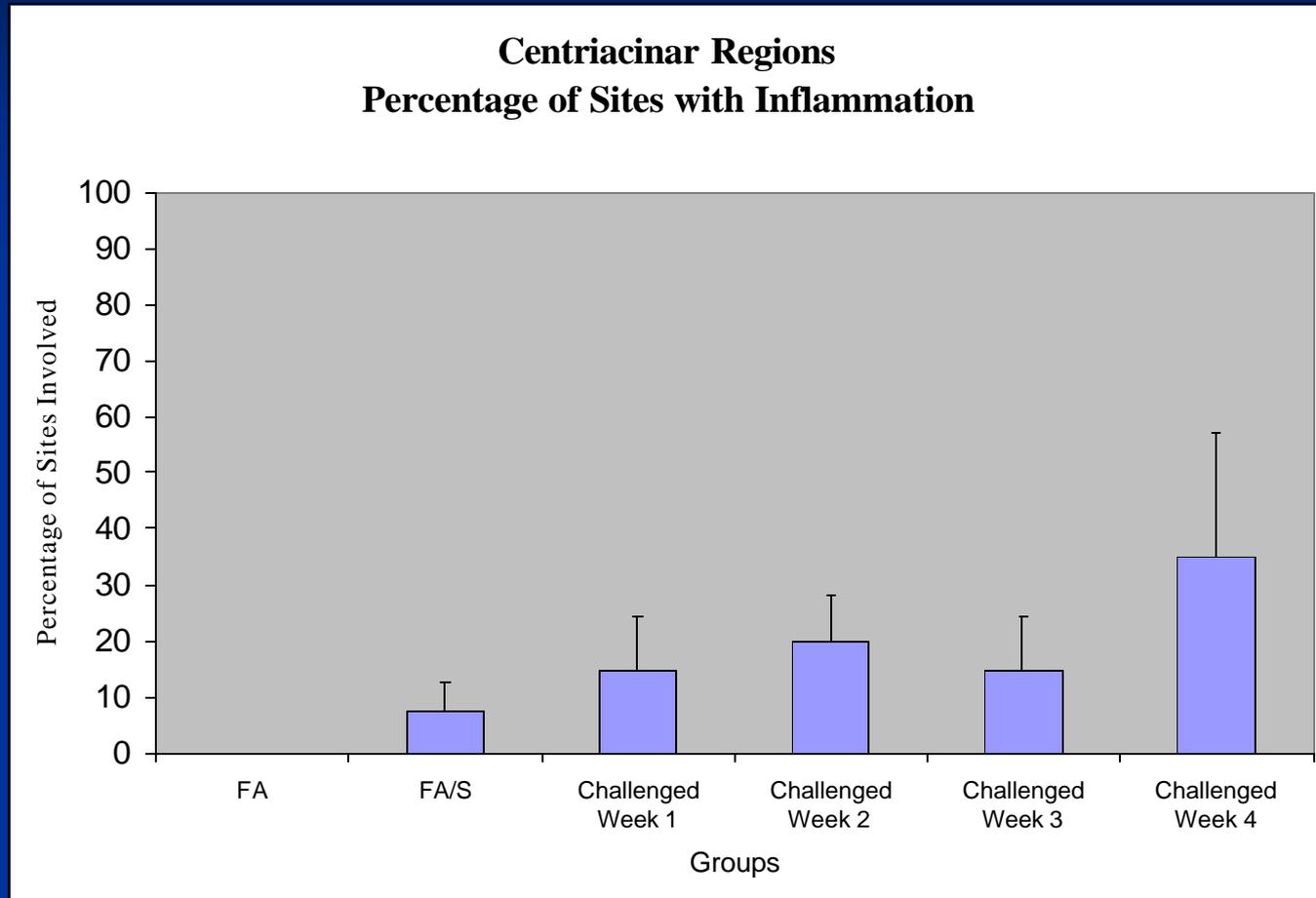


Severe (+++)



Severe (+++)

Centriacinar Regions



$p < 0.05$ compared with FA control

$p < 0.05$ compared with FA/S control

Study Objective Three

- To determine if exposure to PM will alter airway reactivity, lung inflammation, the immune response and epithelial injury in Brown Norway rats sensitized and challenged with OVA.

Hypotheses

- OVA-specific serum IgE and eosinophilic inflammation will be increased in an allergic model and may be changed by PM exposure immediately following allergen challenge
- Detection of a PM effect on airway reactivity and cell permeability may be enhanced in a Brown Norway rat model of allergic airway disease
- Ammonium nitrate and carbon are the two most prevalent forms of PM in California

Exposure

Day 0

Day 14

Exposure

(6 hr/day x 2 days)

Sensitization
(OVA subQ)



Challenge
(OVA aerosol)



FA



PM

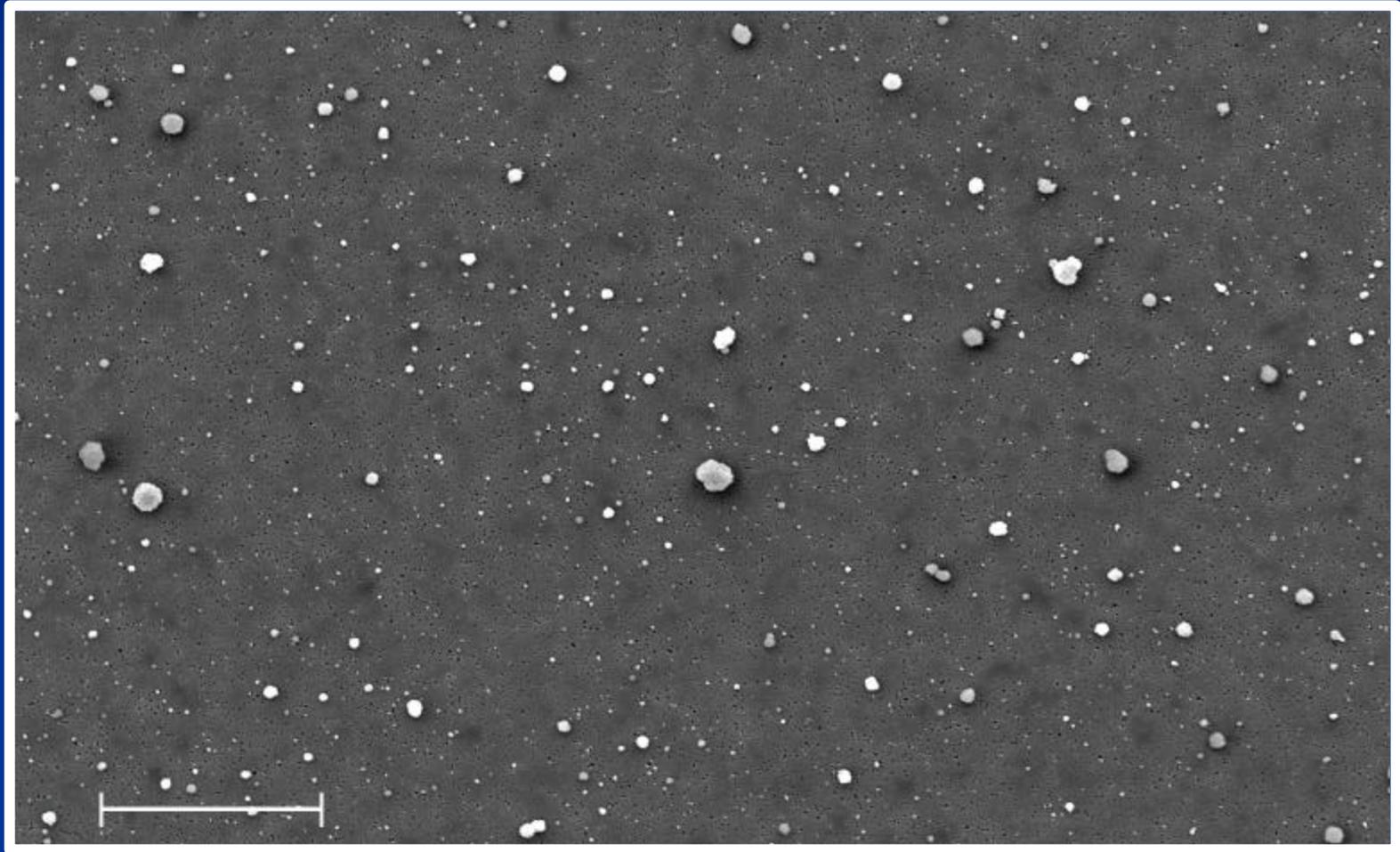
Assays

- Serum IgE: OVA-specific
- PFT: Airway responsiveness (MC)
- BAL: Protein, total cell number, and differential
- Cell permeability: Ethidium homodimer-1
- Cell proliferation: BrdU

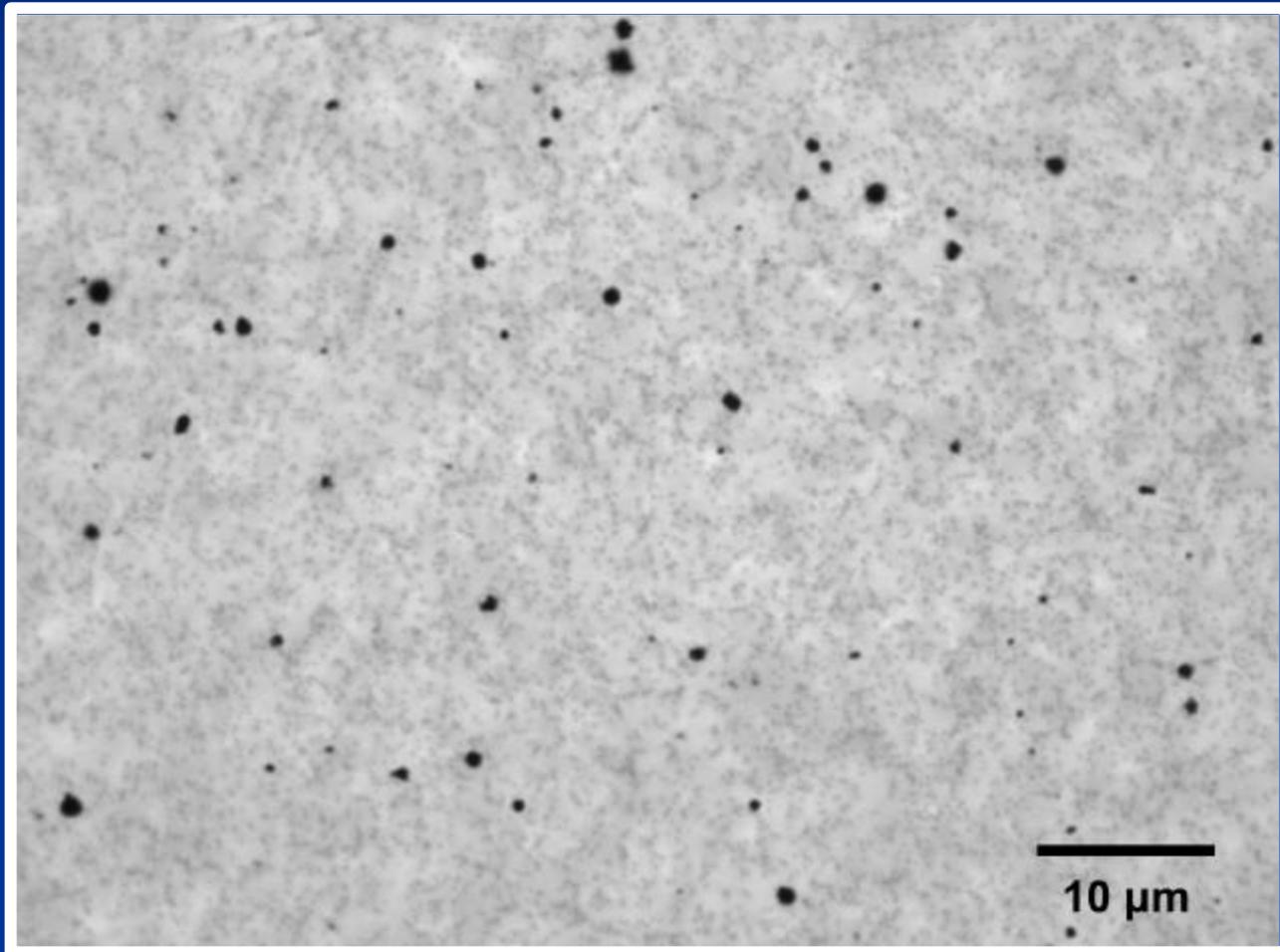
PM Composition

- Ammonium nitrate 150 $\mu\text{g}/\text{m}^3$
- Carbon black 100 $\mu\text{g}/\text{m}^3$
- Particle size (MMAD) 1.0-1.5 μm

Photomicrograph of Collected Ovalbumin Particles



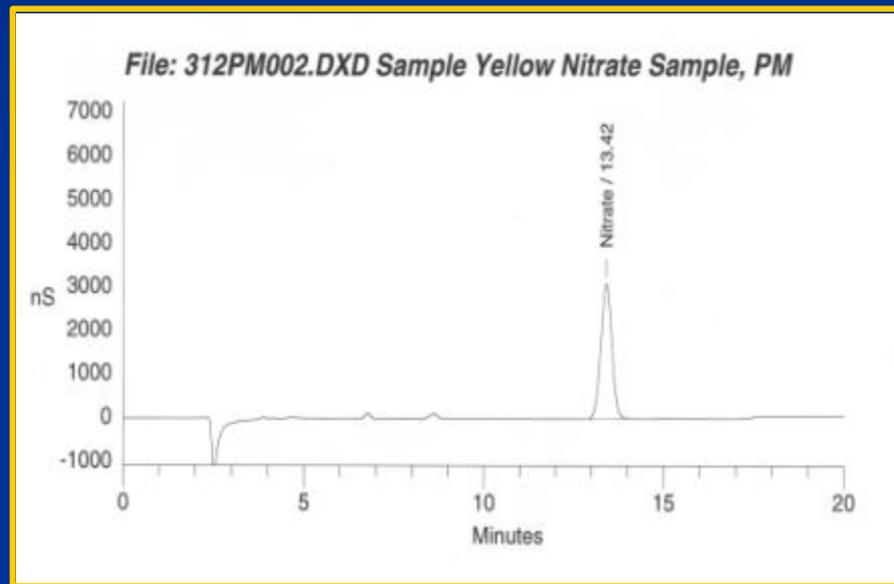
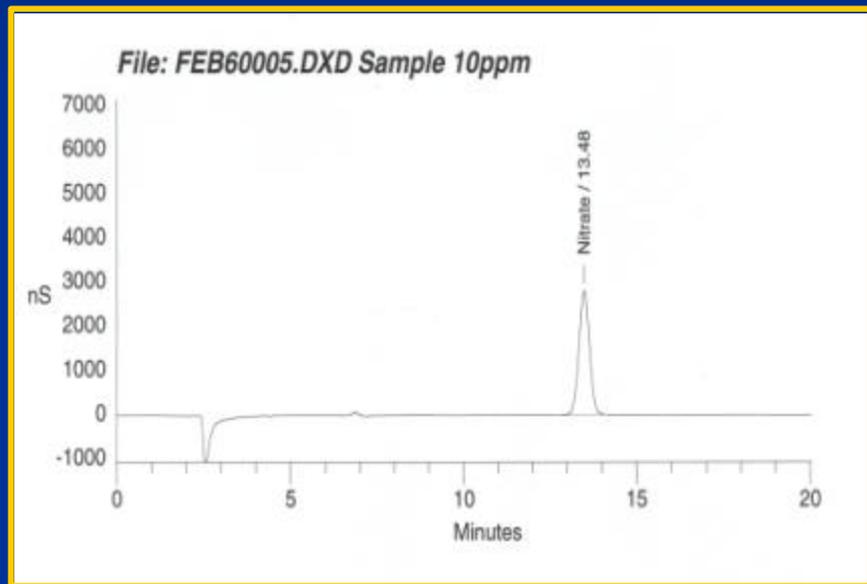
Photomicrograph of Collected PM Particles



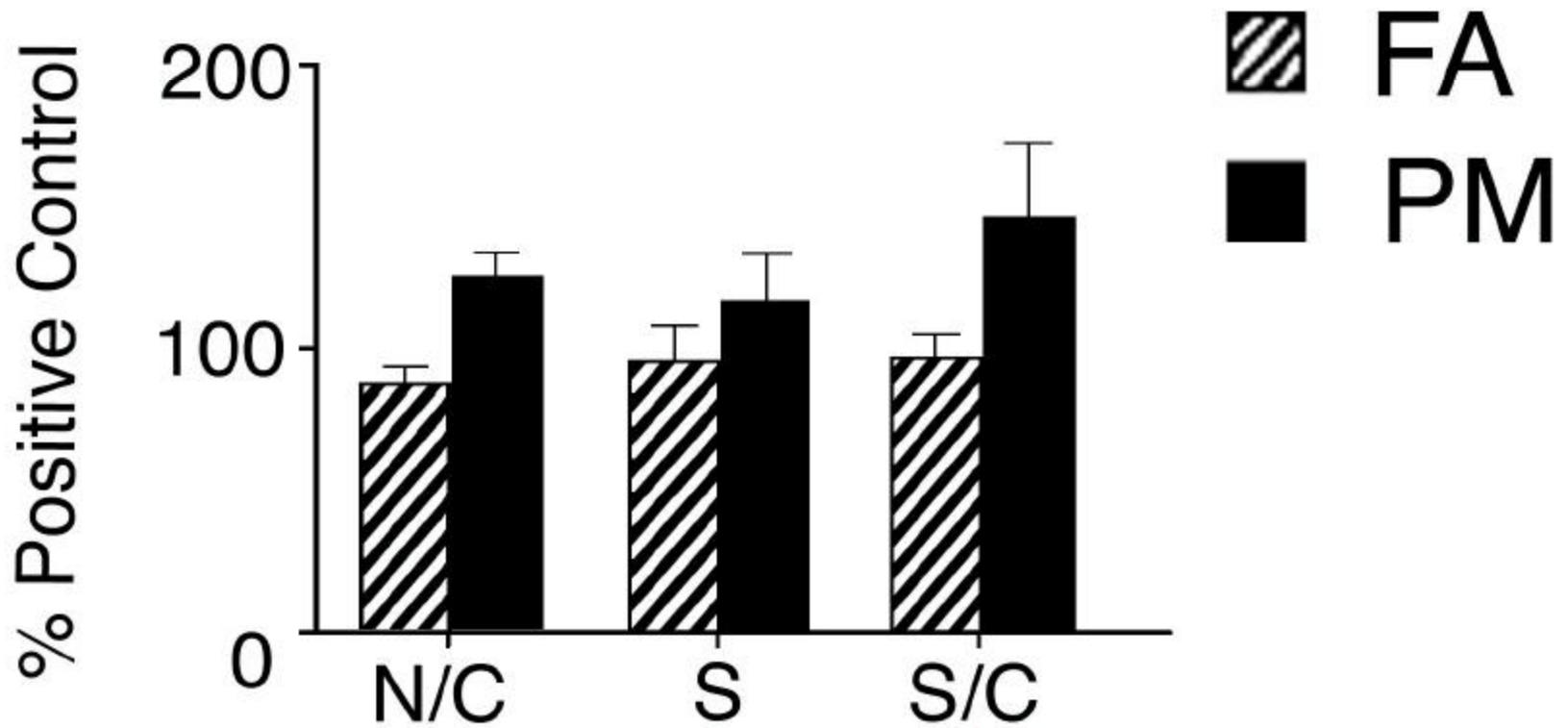
PM Samples for Carbon Analysis



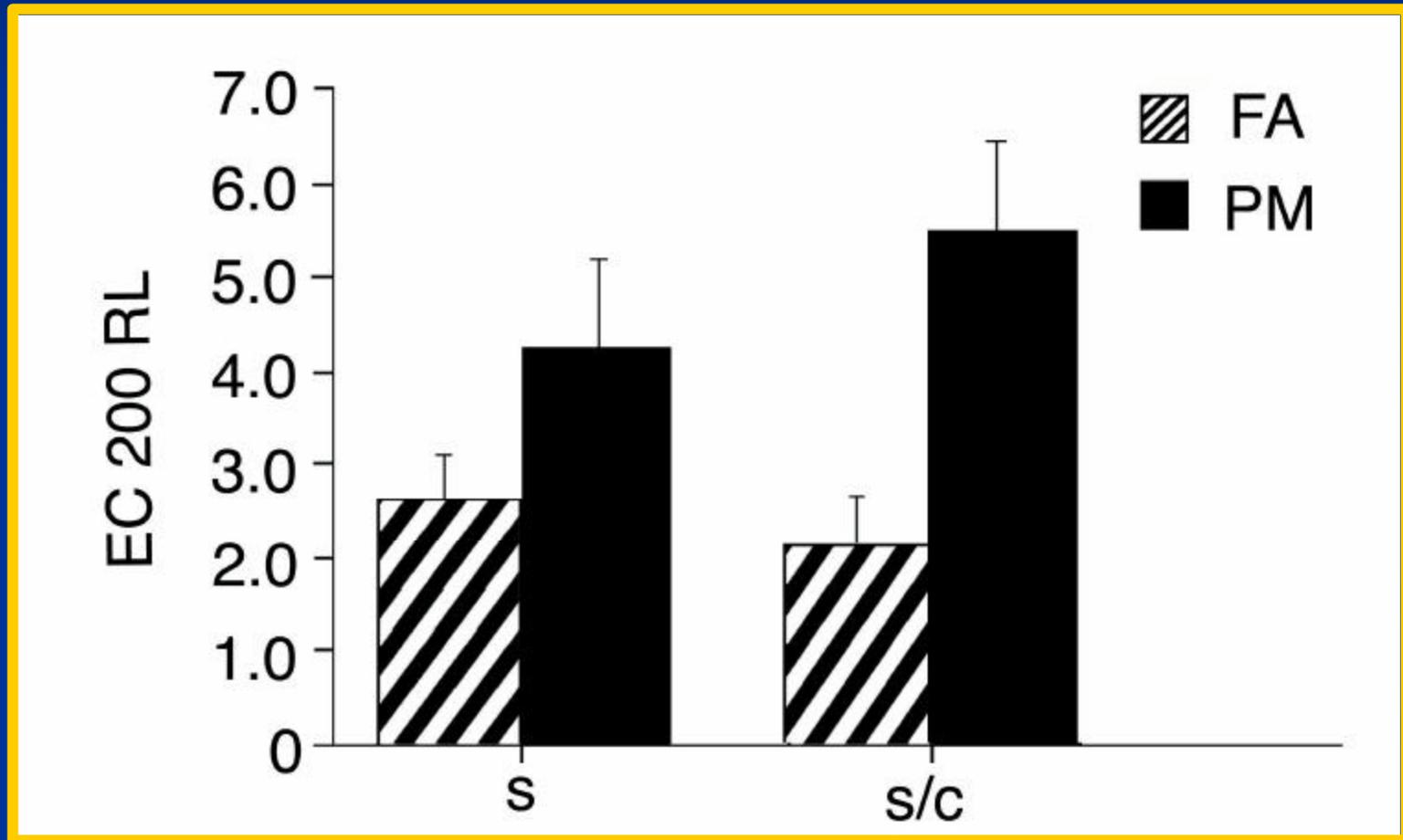
Ion Chromatograms of PM Particle Samples during exposure PM 41



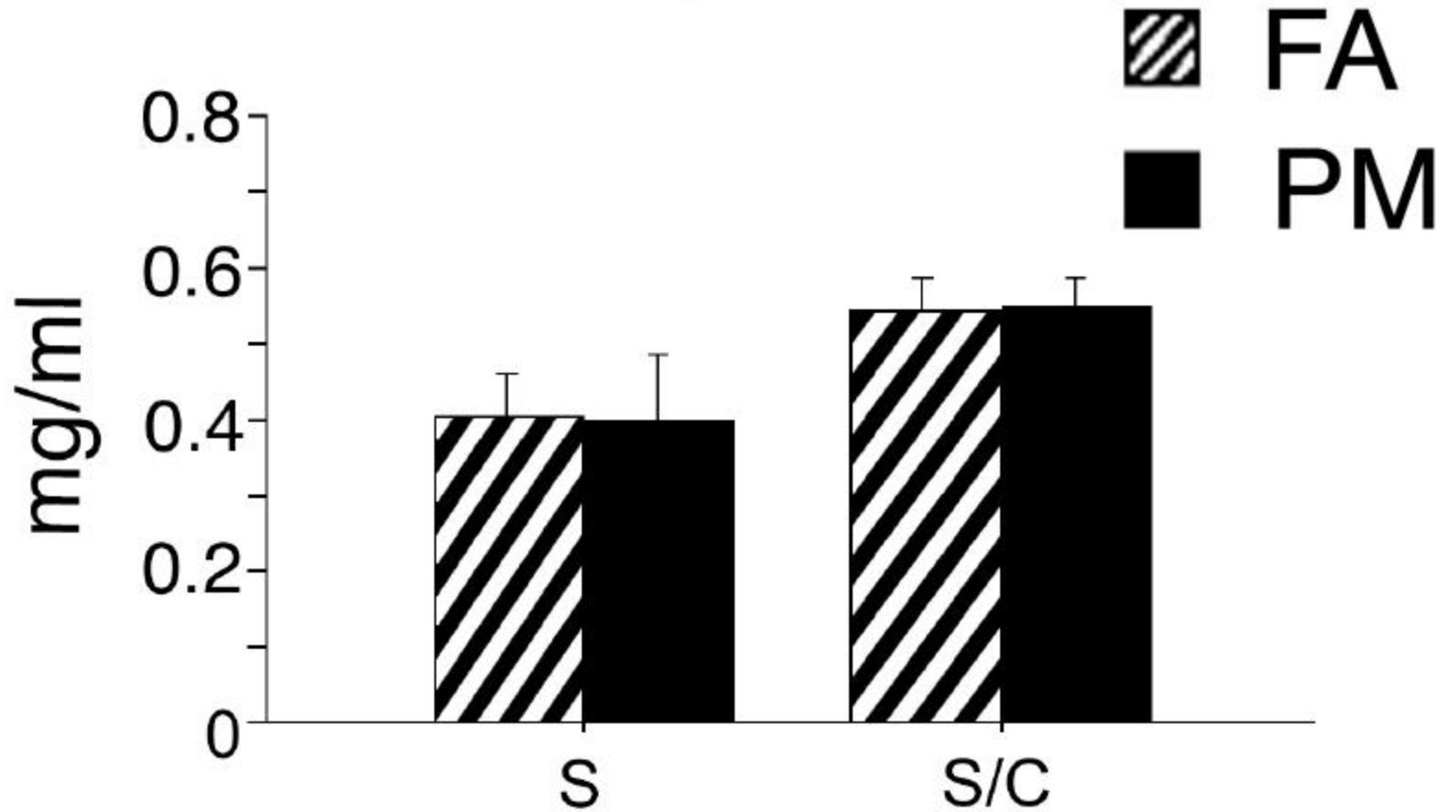
OVA-Specific Serum IgE



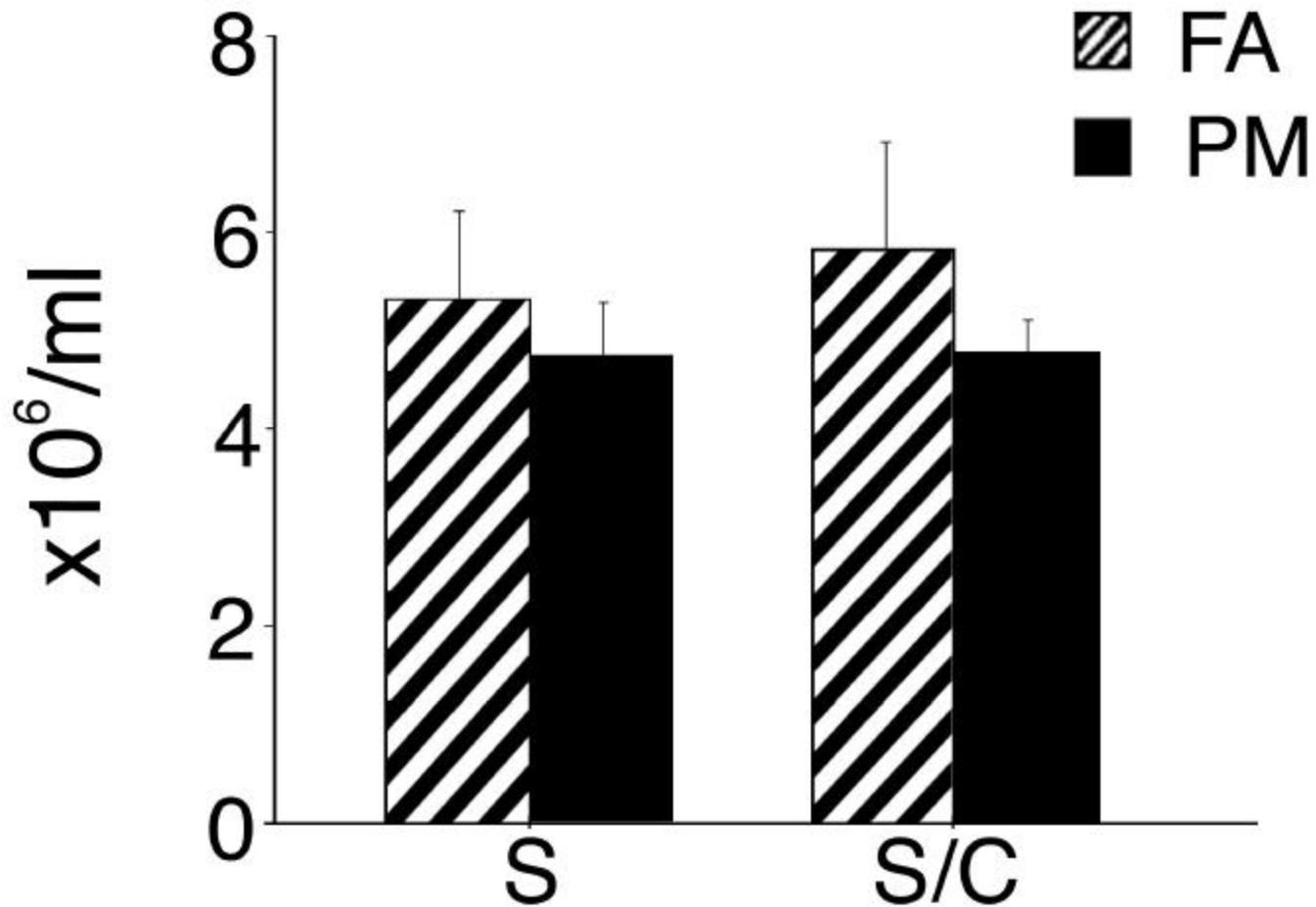
Pulmonary Airway Responsiveness



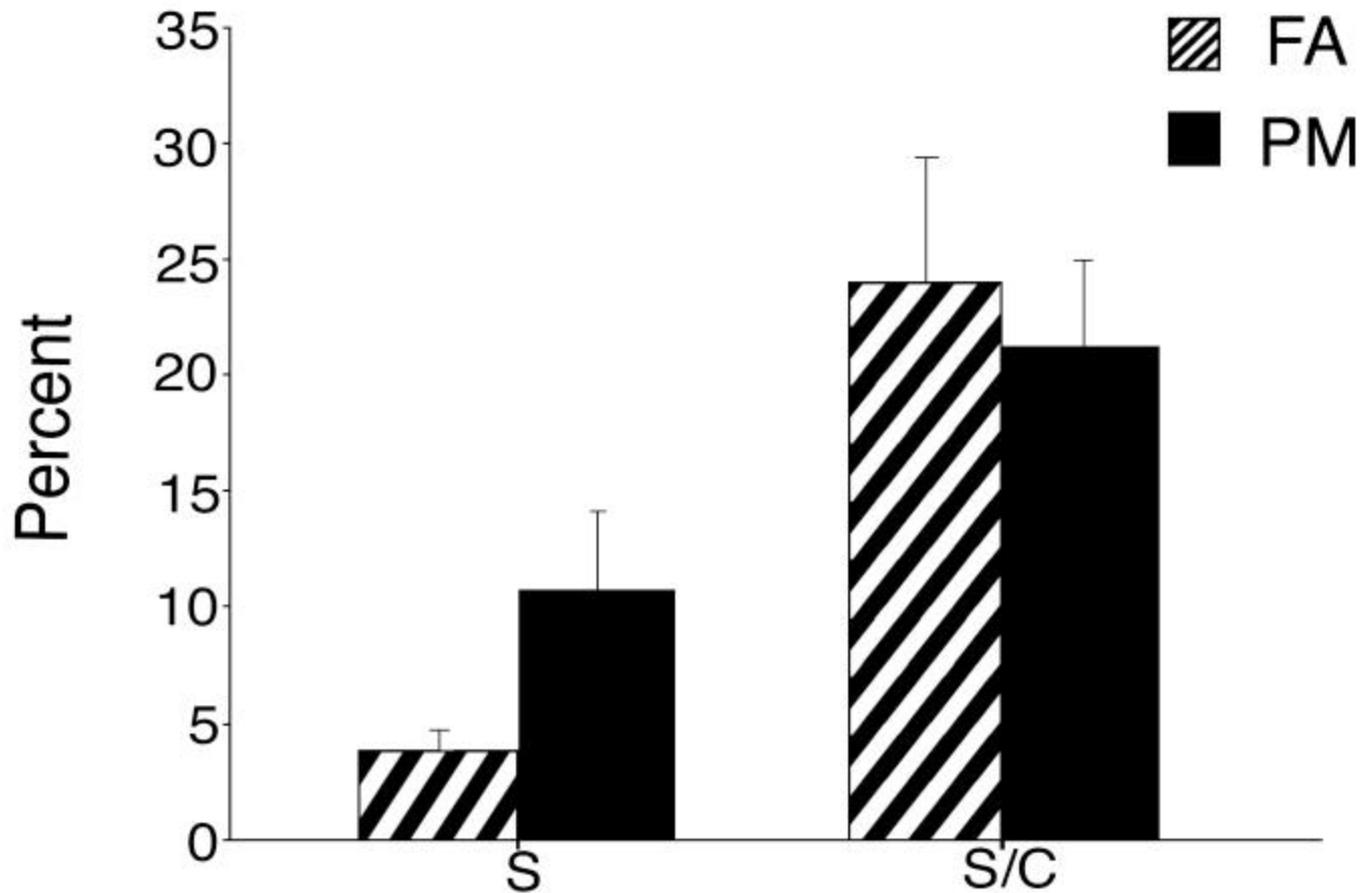
Protein in BAL



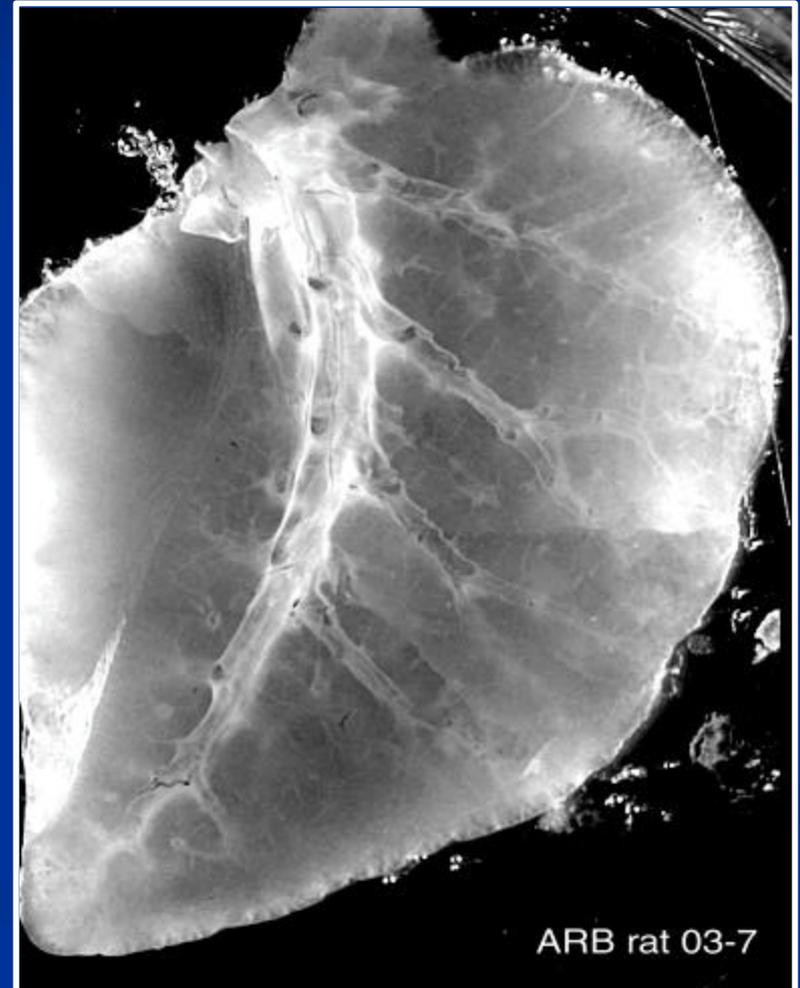
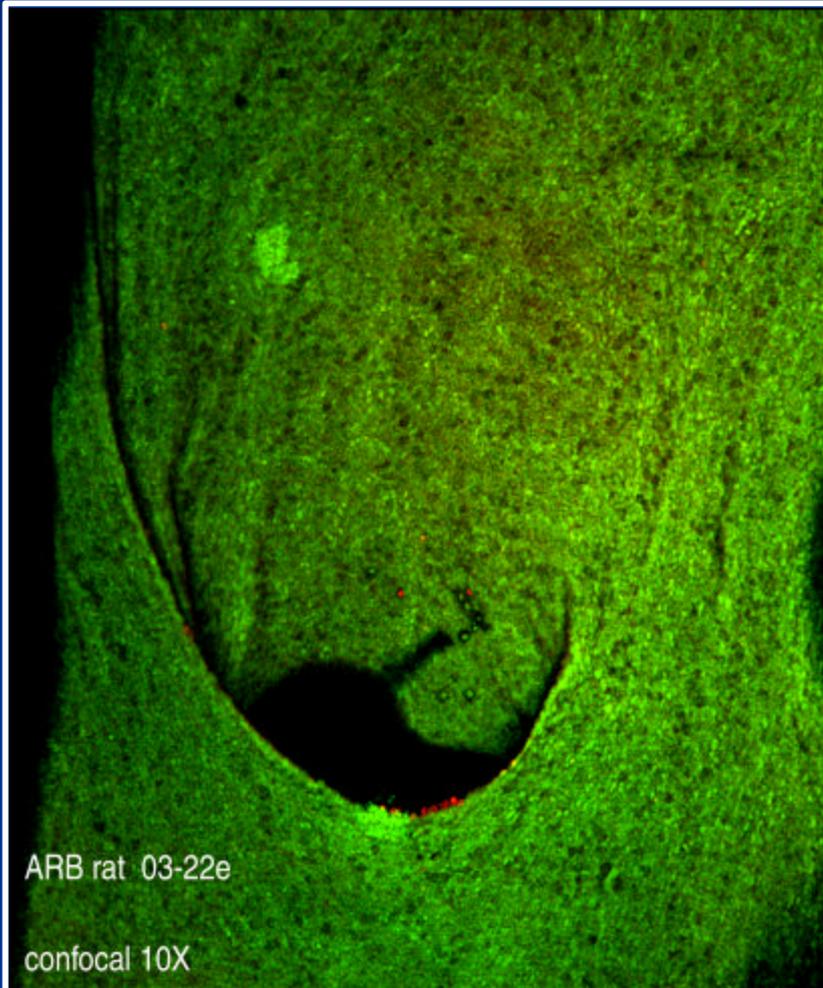
Total Cells in BAL

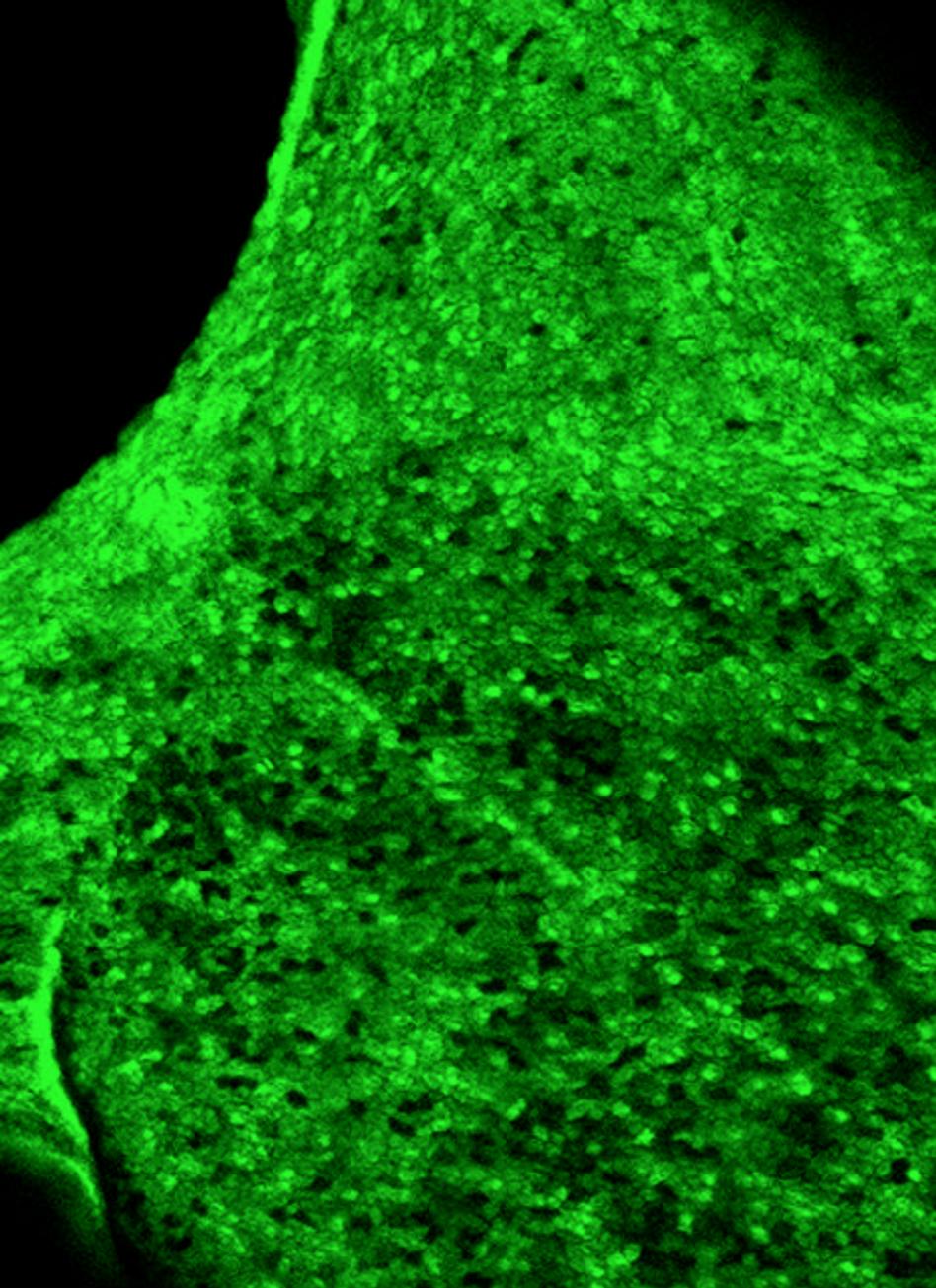


Eosinophils in BAL

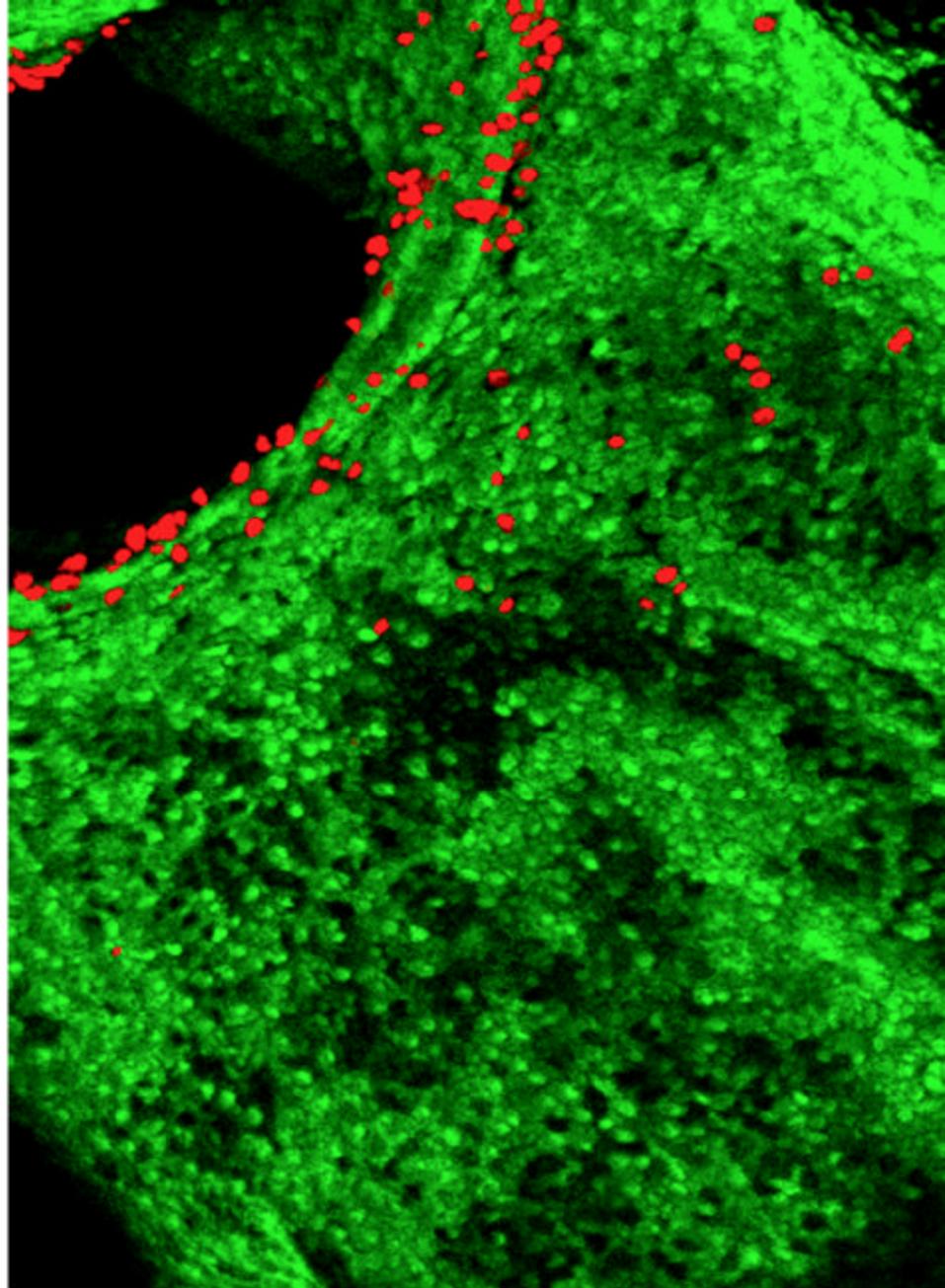


Epithelial Cell Permeability



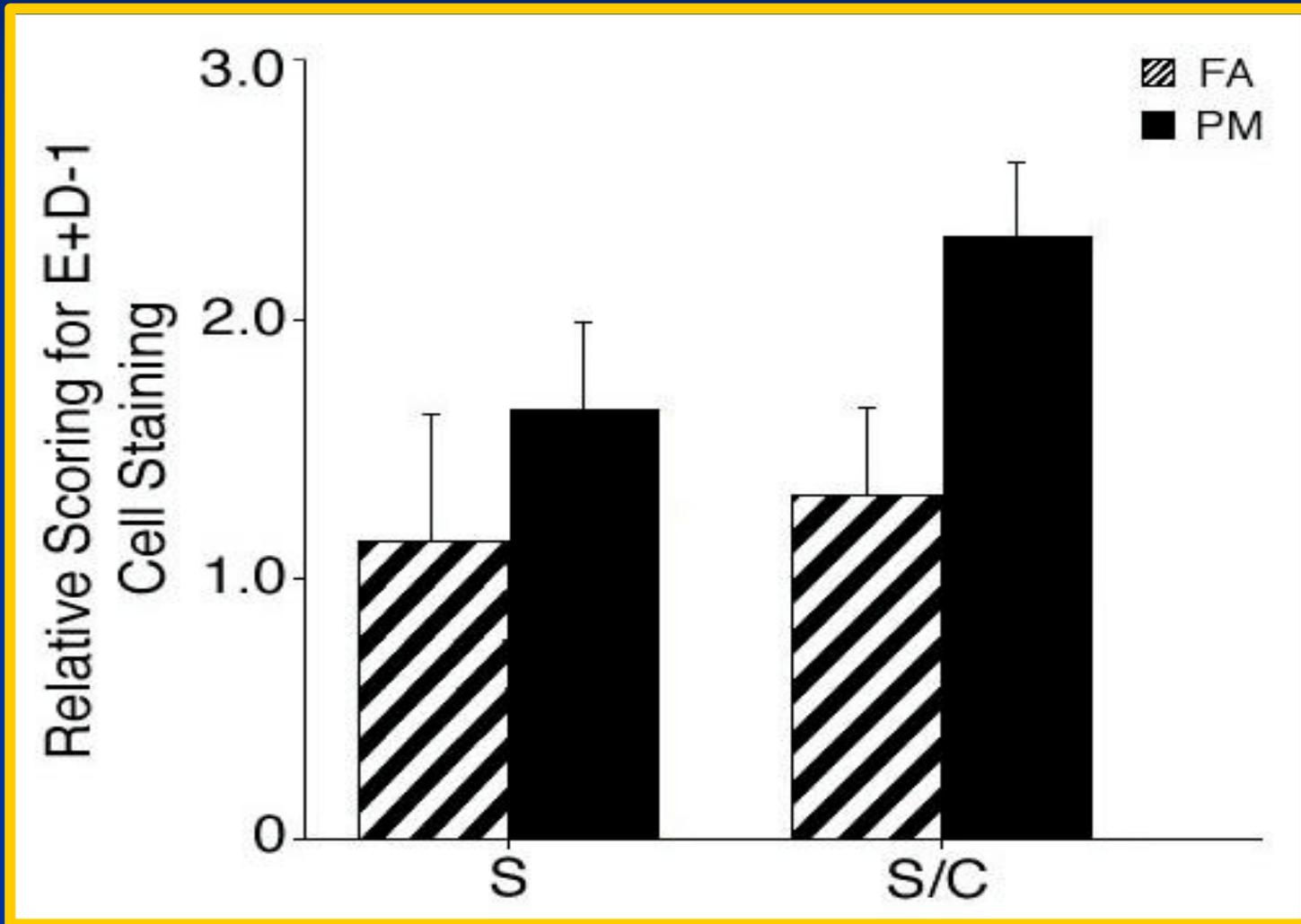


Filtered Air

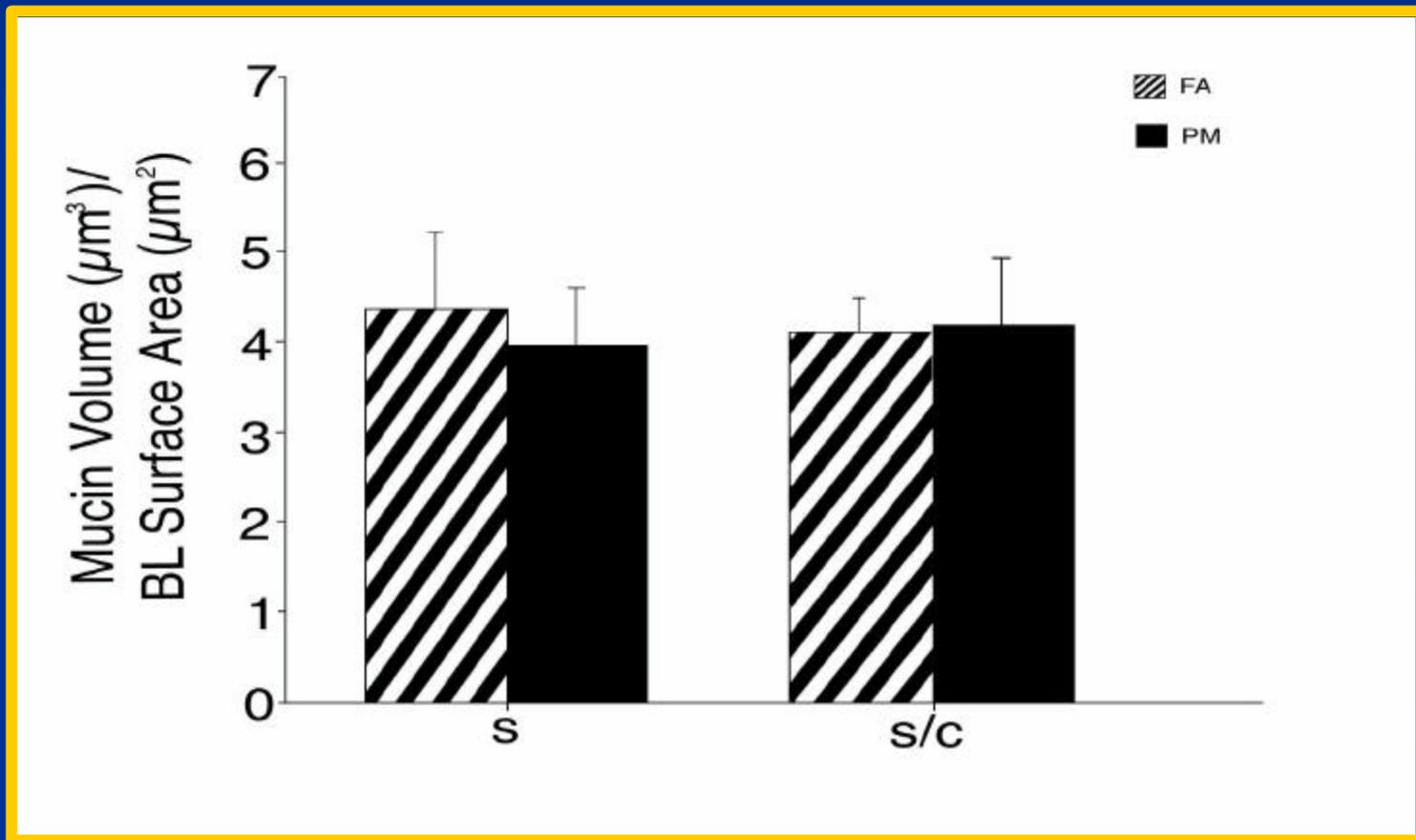


PM

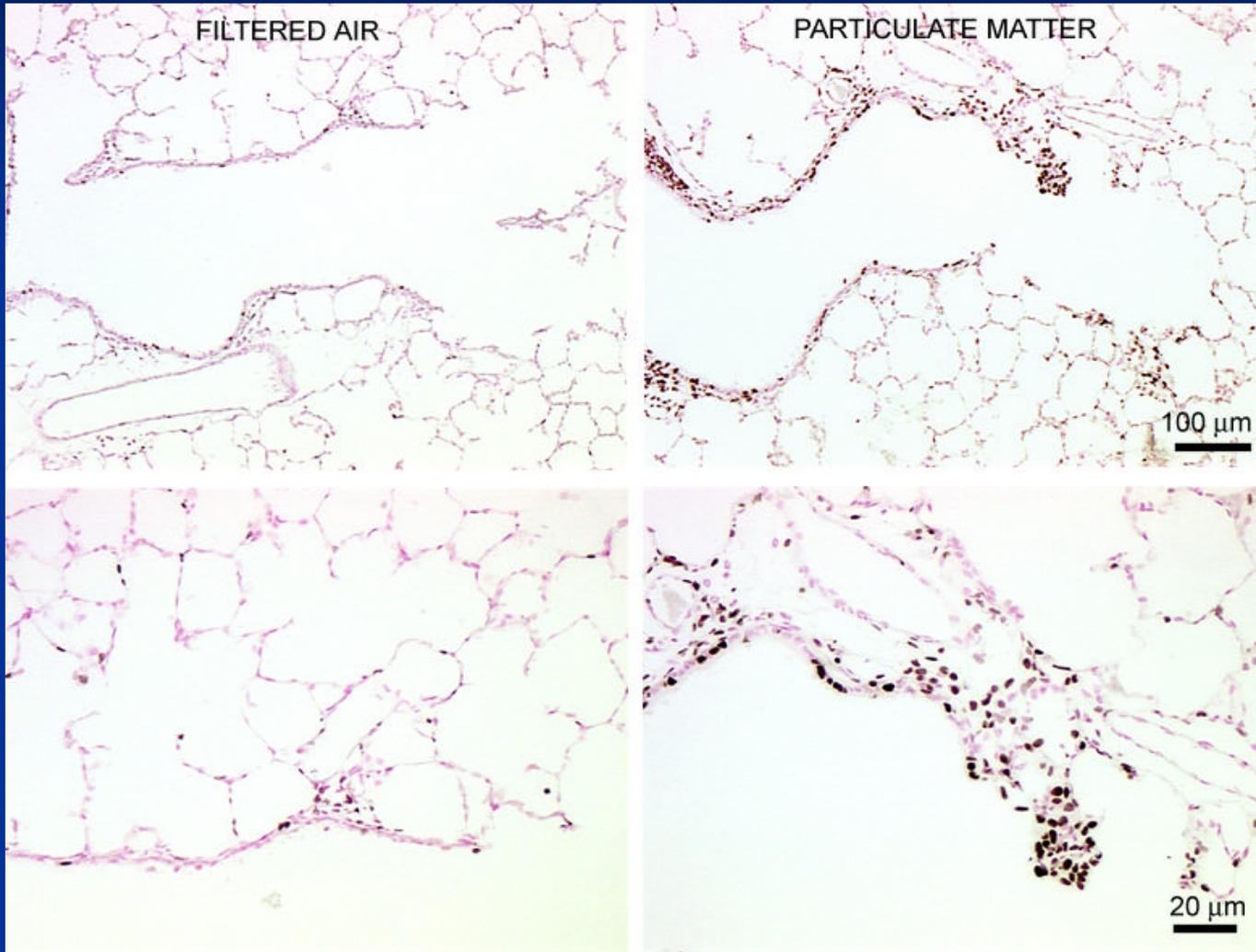
Cell Permeability



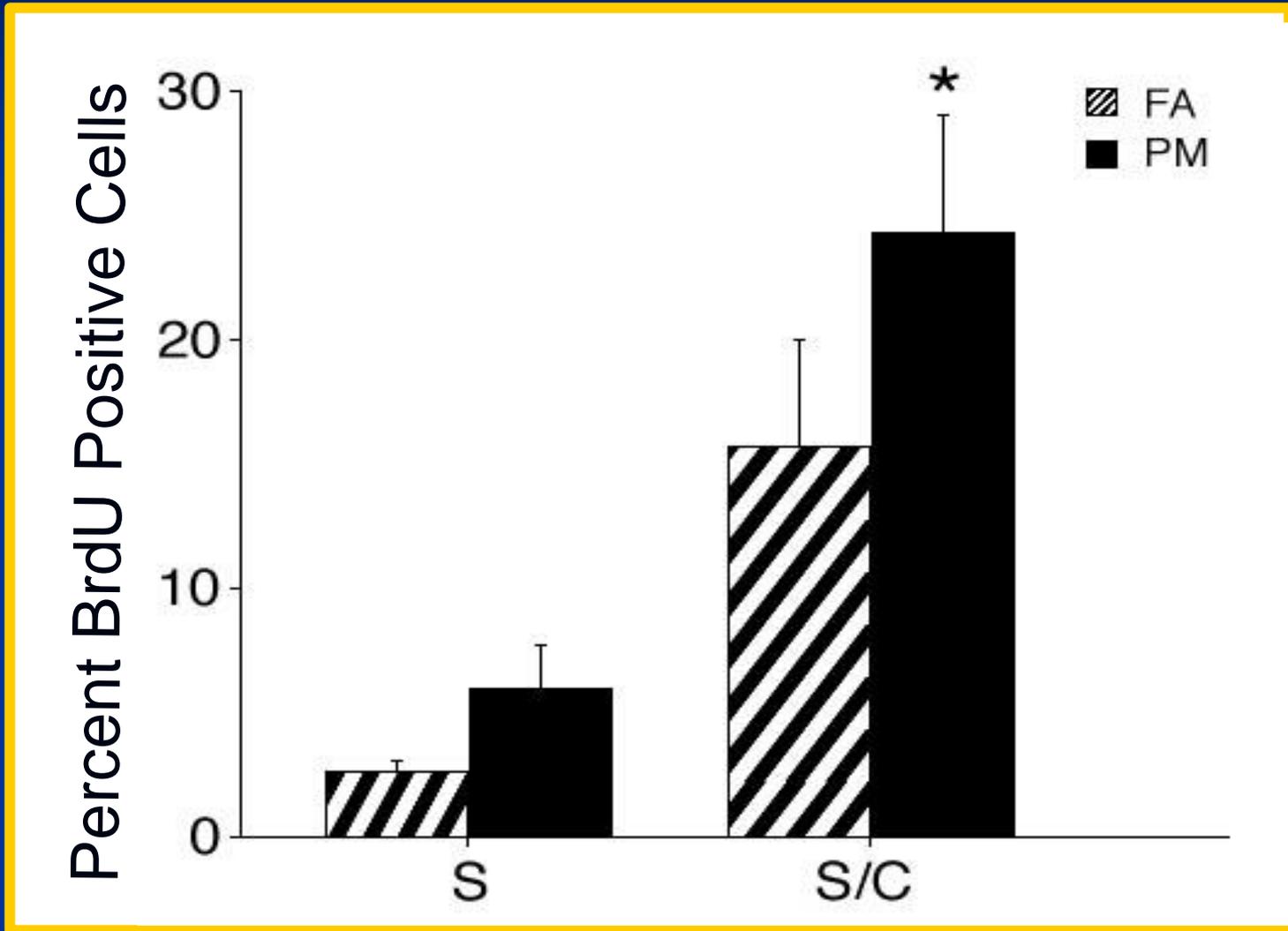
Mucin Volume of Airway Epithelium



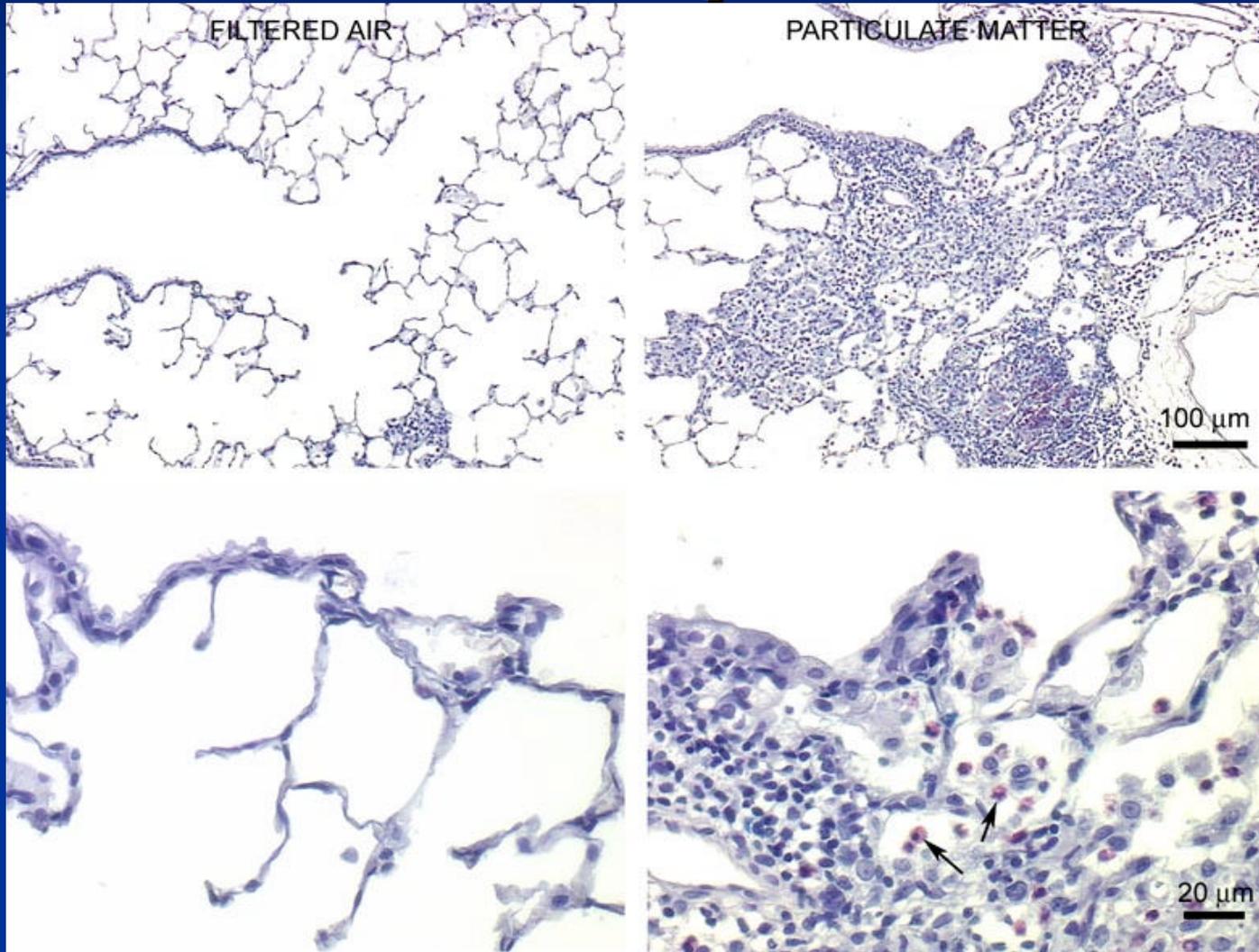
Epithelial Cell DNA Synthesis



Airway Epithelial Labeling

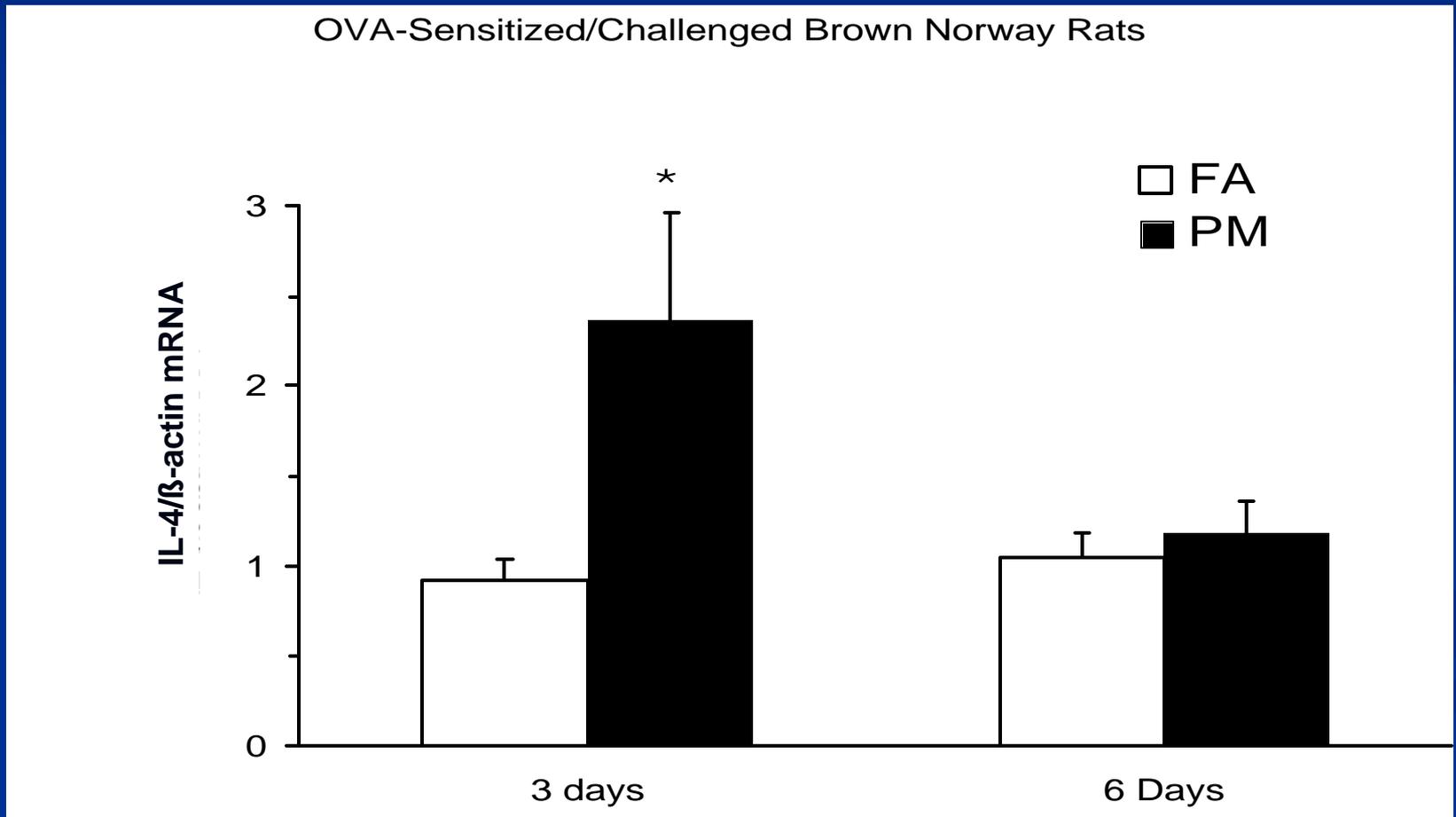


Histological Analysis, Eosinophils





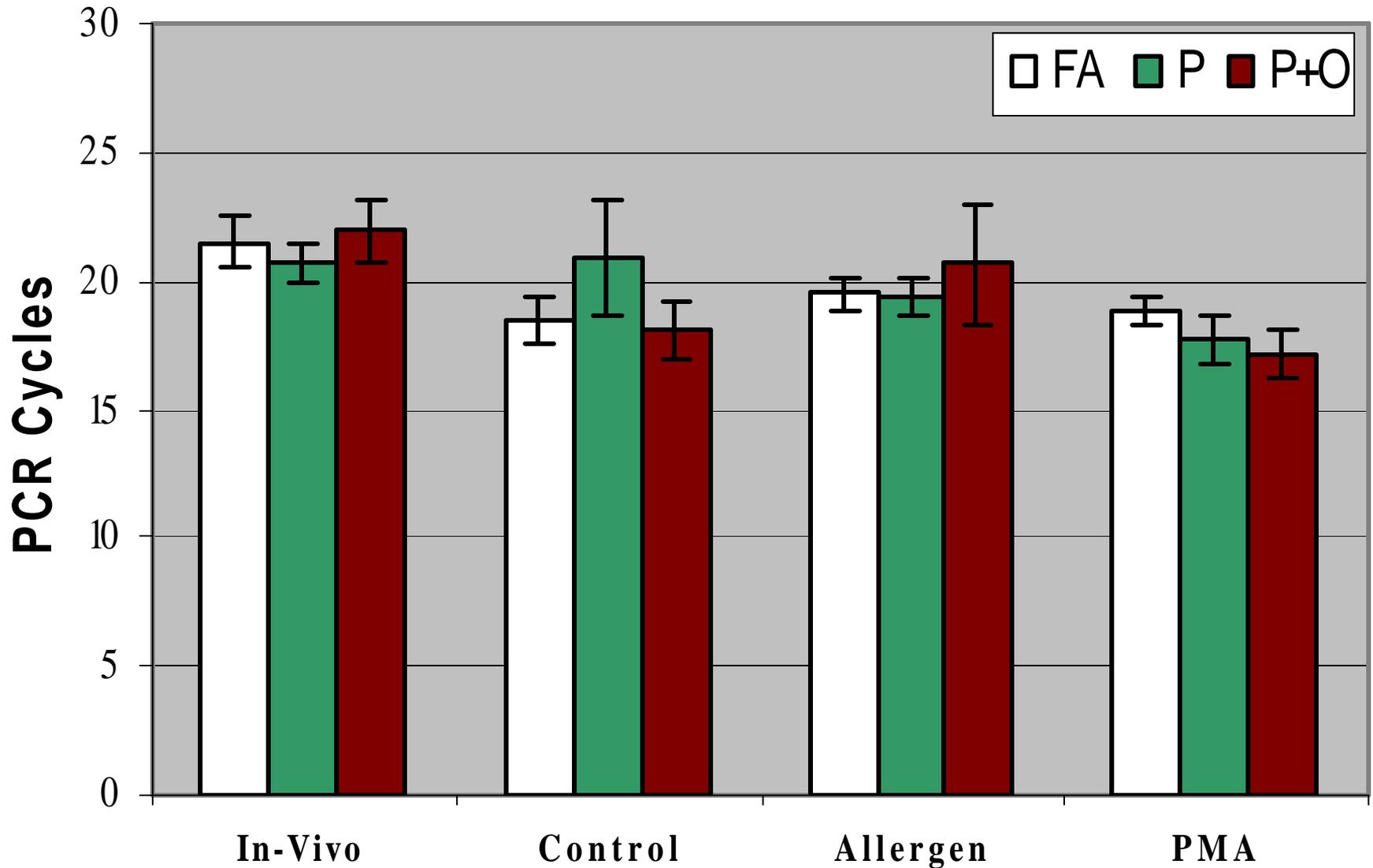
mRNA Levels in Lung Tissues of BN Rats Exposed to FA or PM



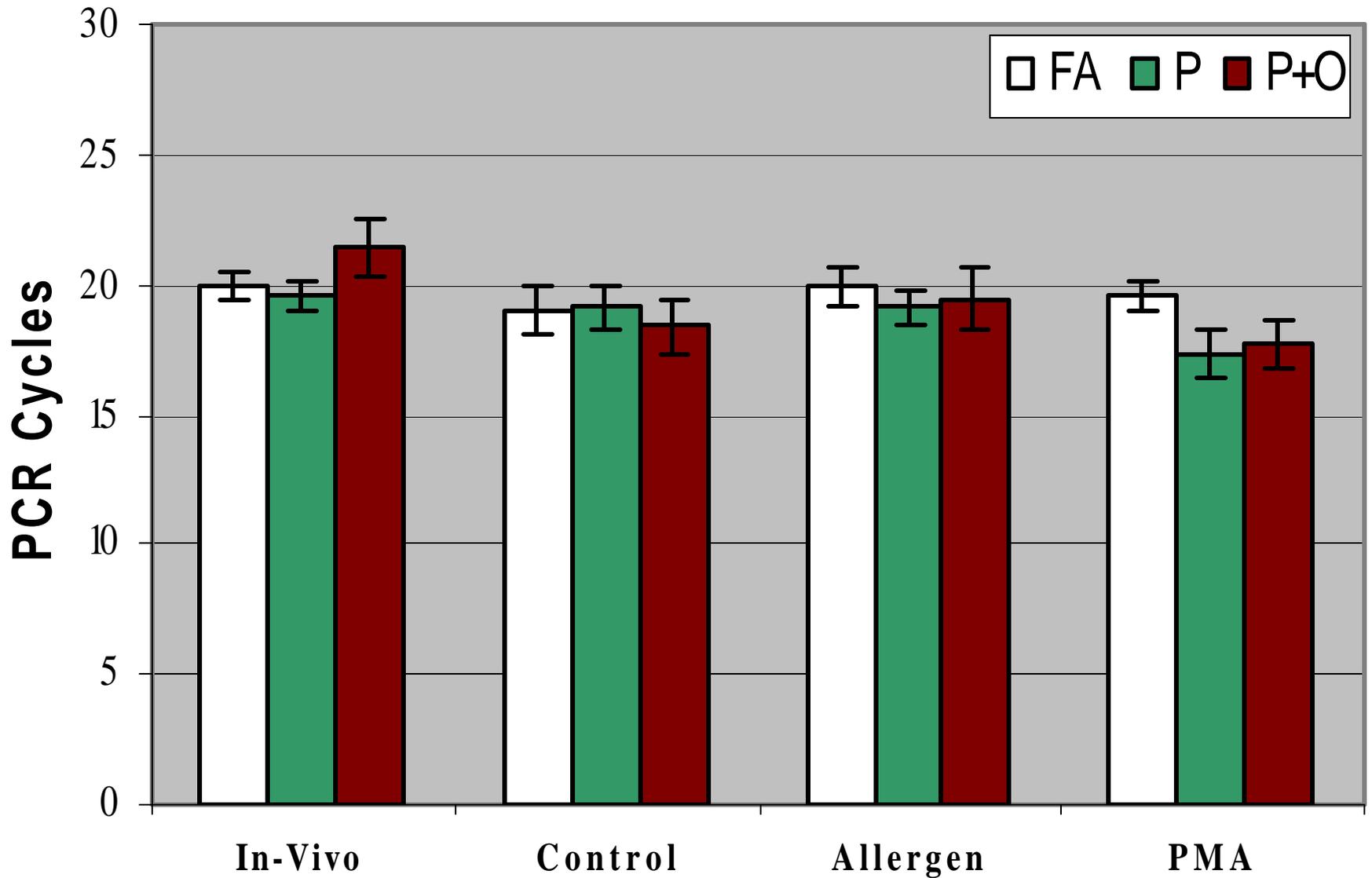
Study Objective Four

- To determine the effects of exposure to PM in human asthmatic volunteers on airway biopsy tissue gene expression for a panel of cytokines.

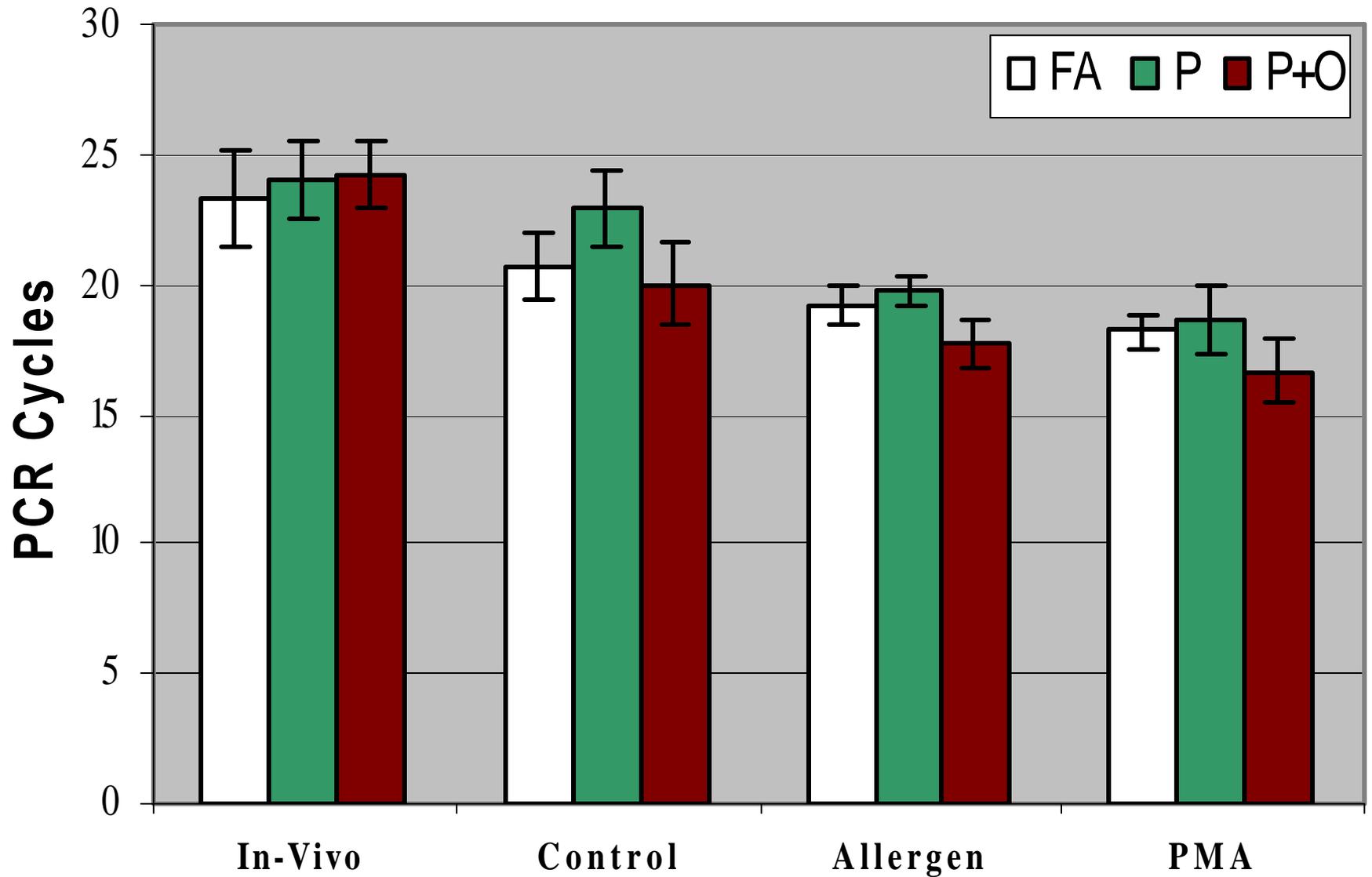
Tissue: IL-12p35 mRNA



Tissue: IL-15 mRNA



Tissue: IL-10 mRNA



Conclusions

- Brown Norway rats can serve as a reasonable model of allergic airway disease following a single sensitization and challenge with OVA
- PM may serve as an adjuvant and increase systemic levels of IgE following sensitization and challenge in Brown Norway rats
- Airway hypersensitivity was not changed by sensitization and challenge in this model. Exposure to carbon and ammonium nitrate PM may actually decrease response to MC challenge

Conclusions

- Airway epithelial cell permeability to ethidium homodimer-1 in our model may increase following two days of exposure to carbon and ammonium nitrate particles (N.S.)
- Significant increases in airway epithelial cell BrdU labeling occur in this model of allergic airway disease following exposure to carbon and ammonium nitrate PM ($p < 0.05$)
- Centriacinar alveolitis may be increased by PM exposure in a Brown Norway rat model of allergic airway disease (N.S.)

Conclusions

- Human airway biopsies can be used to examine gene expression for the variety of different cytokines
- Gene expression of cytokines within human airways is not significantly altered following acute exposure to carbon and ammonium nitrate PM
- IL-5 gene expression in Brown Norway rats is transiently altered following acute exposure to carbon and ammonium nitrate PM
- Combined animal and humans studies can be used to provide new insights on potential mechanisms of the health effects of PM on the respiratory system

Acknowledgements

- Ed Schelegle
- Charles Plopper
- Laurel Gershwin
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- Maria Suffia
- Julian Recendez
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Exposure

