Inflammatory Processes in a Mouse Model of Chronic Pulmonary Diseases by Cigarette Smoke Inhalation

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Presentation Outline

- Background and research need
- Program objective, study design
- Disease model
  - Emphysema
  - Lung tumors
- Mechanistic evaluations
  - Gene expression (in non-tumor lung tissue, hypothesis-driven evaluation)
  - Inflammatory markers in bronchoalveolar lavage (BAL)
- Summary and conclusion

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Background and Research Need

- Smoking is a cause of multiple severe chronic diseases, such as lung cancer and emphysema.
  - Common and distinct mechanisms suggested
- The 5-year overall survival rate for lung cancer has remained at 15% for several decades
  - Late discovery of tumors in advanced stages
  - High metastatic potential even at early stages
- Need for interventions and improved diagnostics
  - No smoking
  - Biomarkers of disease
  - Therapies
  - Chemoprevention
  - Less risky tobacco products

Improved mechanistic understanding and disease models required

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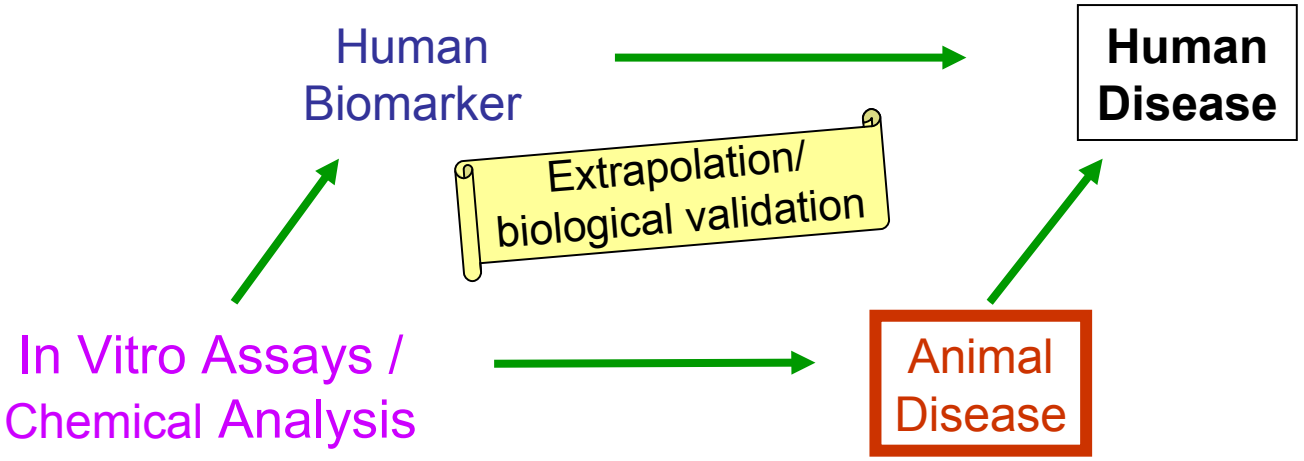
Role of Non-Clinical Disease Models

- Lung cancer: approx. 80% of cases in humans are non-small cell lung cancers, with adenocarcinoma being the most prevalent and increasing in incidence
- Laboratory animal models develop pulmonary adenoma and adenocarcinoma as well as emphysema
- Non-clinical disease models involving chronic smoke inhalation
  - Not widely used
  - No general agreement on study design
  - Limited mechanistic understanding



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Program Objective and Design

- Develop smoking-related non-clinical disease models for lung cancer and emphysema
- Evaluate the role of inflammatory processes

- Animal model: A/J mouse (males)
  - Susceptible for spontaneous and chemically induced pulmonary tumorigenesis (K*ras*-dependent)
  - Reproducible development of lung tumors with an environmental tobacco smoke surrogate (after post-inhalation period only)
  - Reproducible development of emphysema with cigarette mainstream smoke (MS) inhalation (~ 5 to 6 months)
- End points:
  - Histopathology, incl. morphometry
  - gene expression changes in whole lung (without tumors)
  - molecular and cellular inflammatory endpoints in bronchoalveolar lavage fluid

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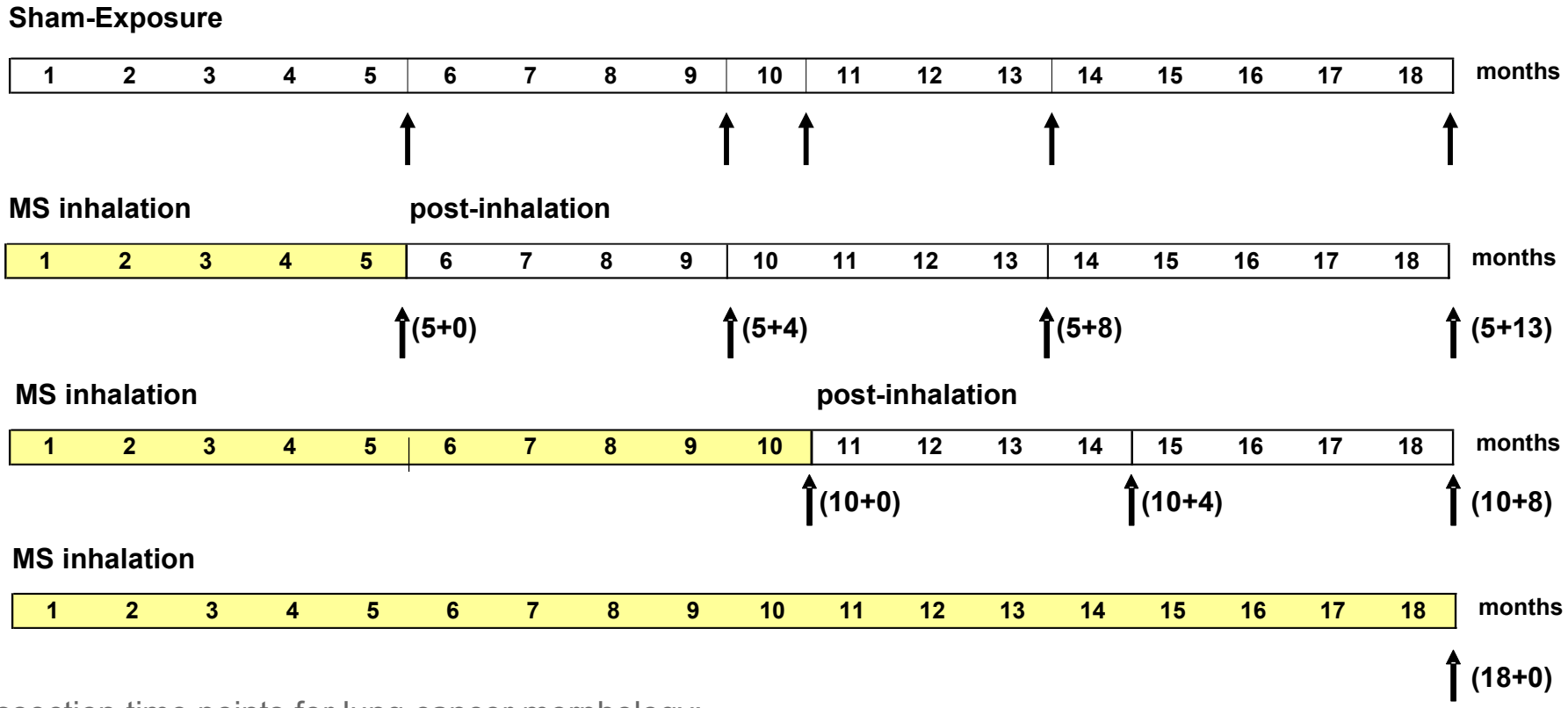
Study Design

Inhalation: 6 hours/day, 5 days/week (whole-body)

Mainstream smoke conc.: 0, 150, and 300 mg TPM/m^3 (sham, MS-150, and MS-300); Reference Cigarette 2R4F



(↑ Dissection time points for lung cancer morphology; similar for other end points.)

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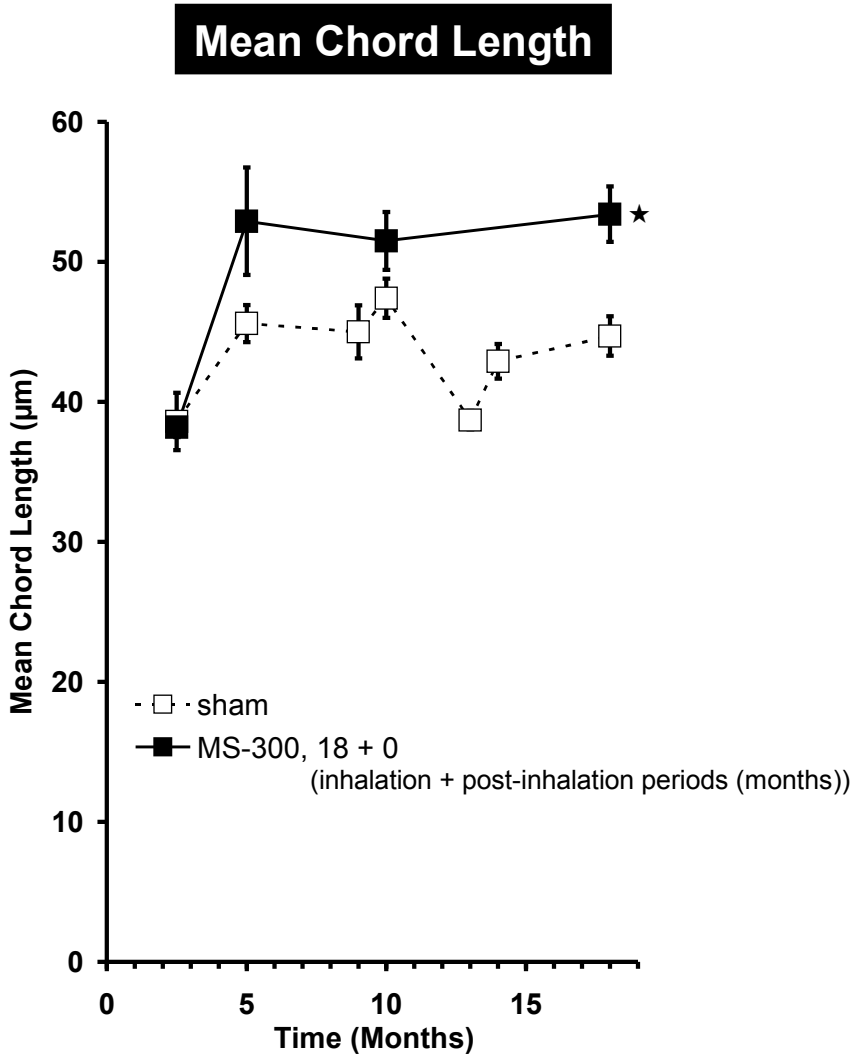
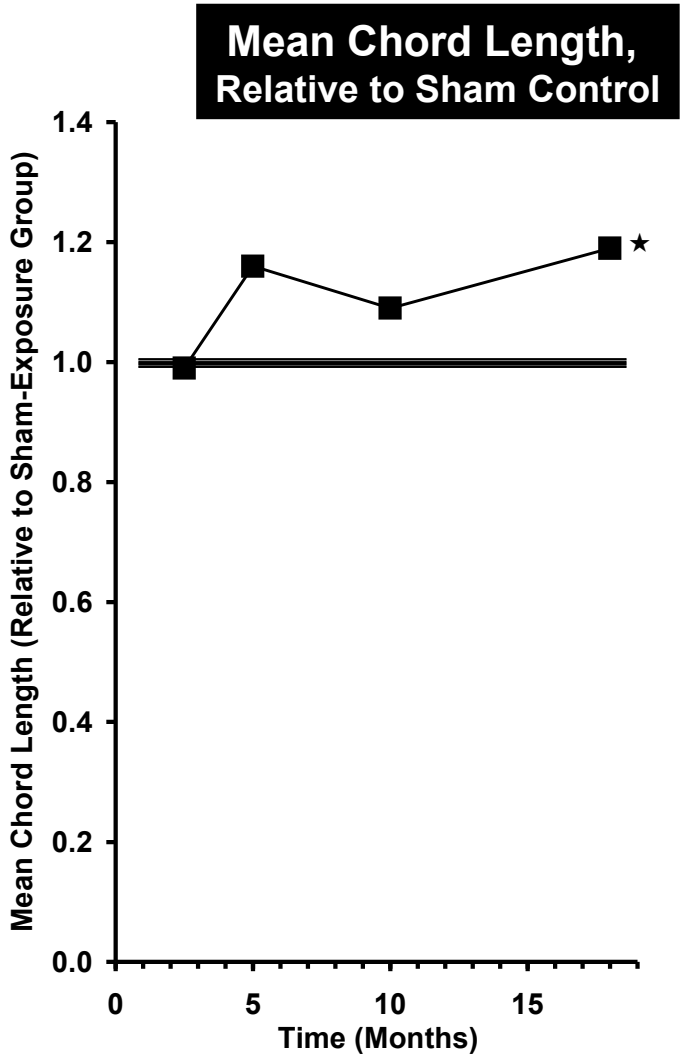
Time Course of Emphysema Development





(N = 6 to 10; mean ± SE; one-way ANOVA with sham, MS-150, and MS-300, followed by Tukey post-hoc test)

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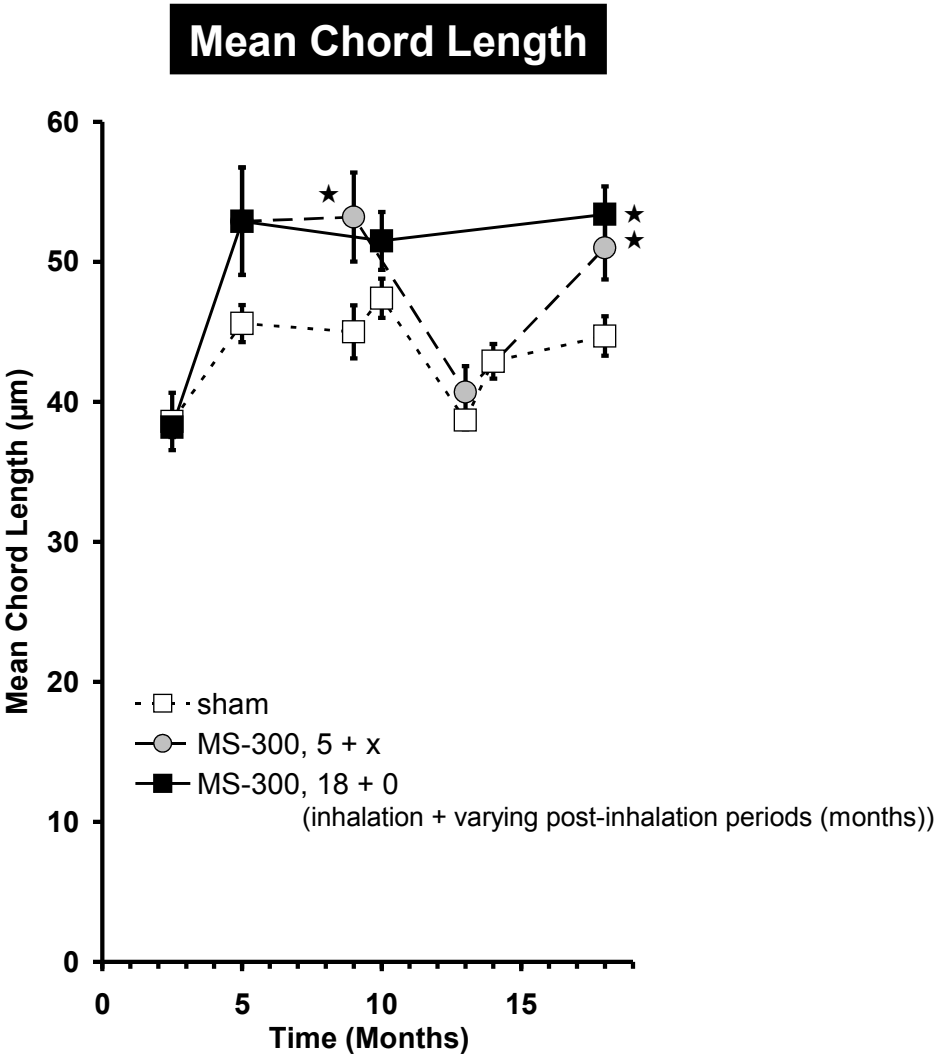
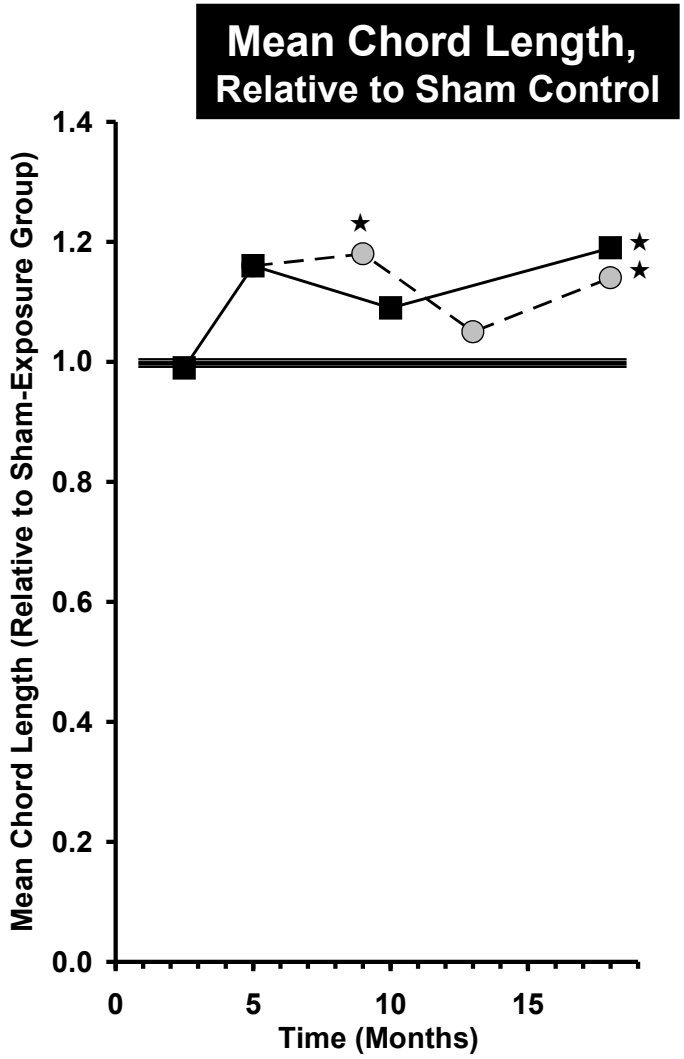
Time Course of Emphysema Development





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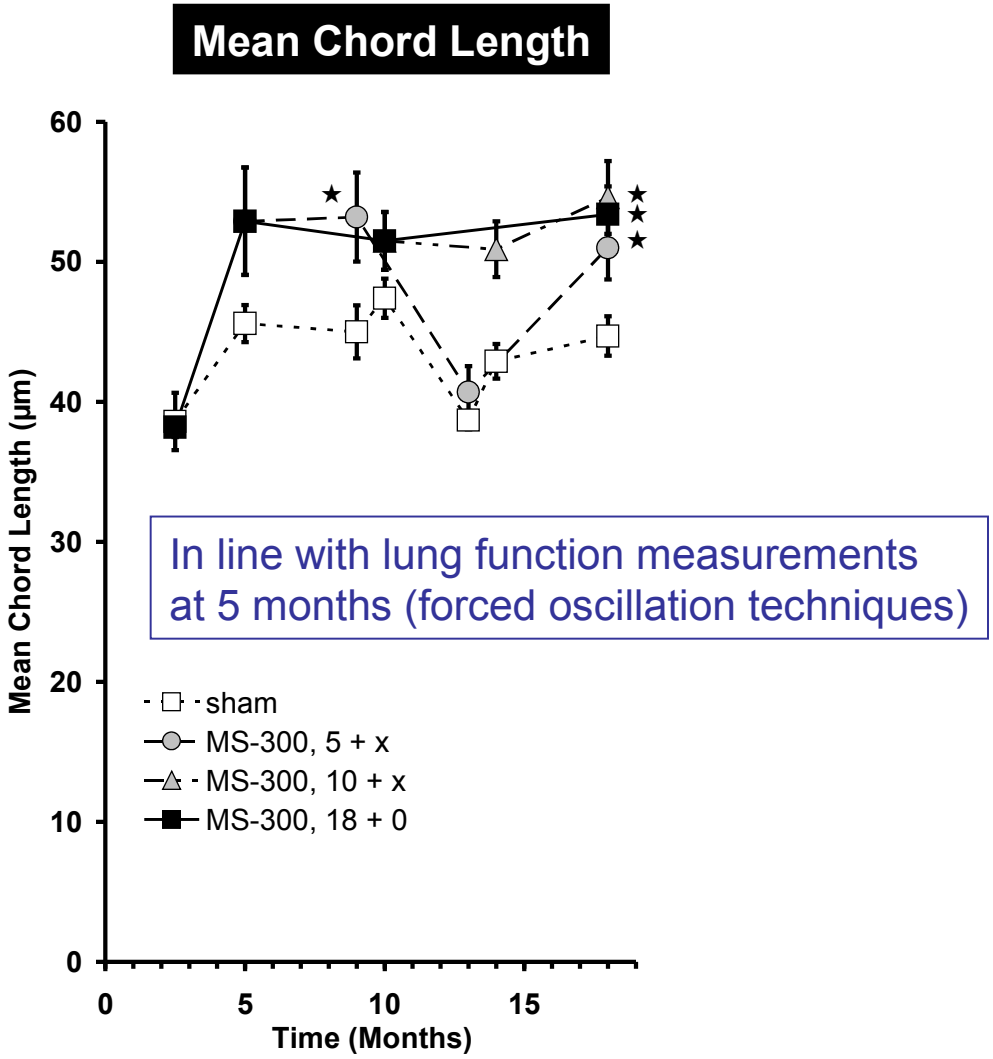
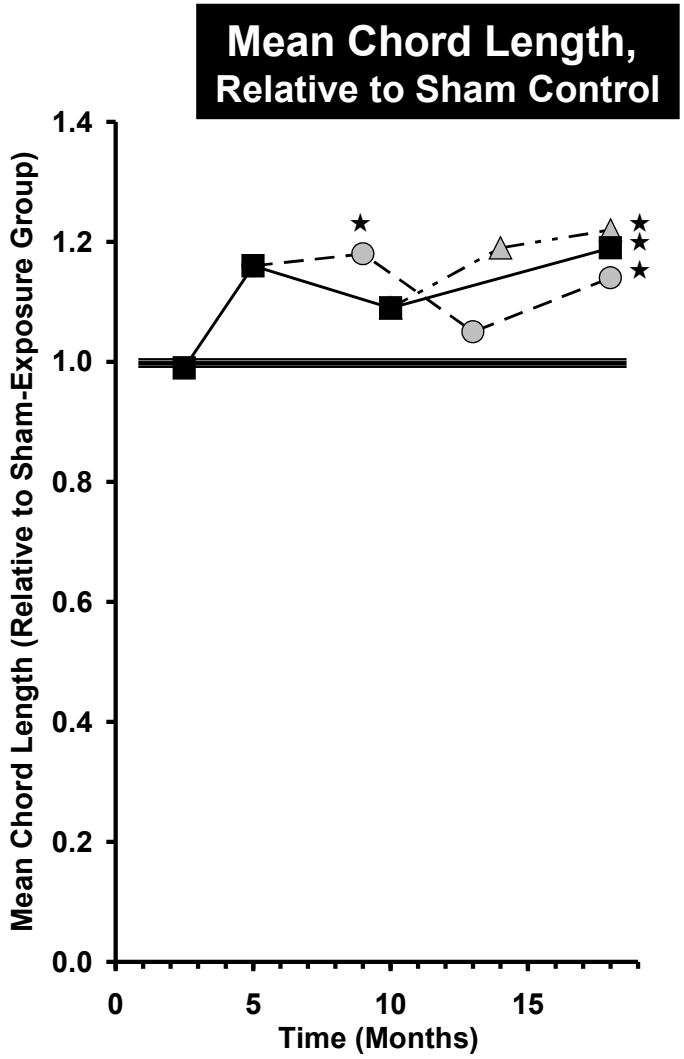
Time Course of Emphysema Development





(N = 6 to 10; mean ± SE; one-way ANOVA with sham, MS-150, and MS-300, followed by Tukey post-hoc test)

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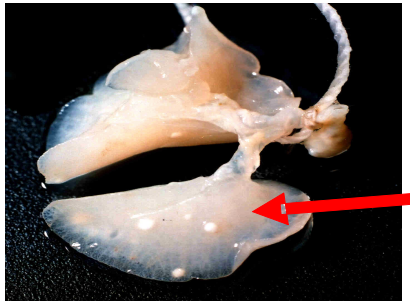
Lung Tumor Formation

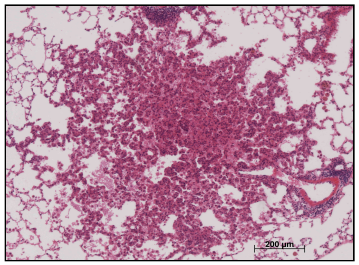


Lung Nodules

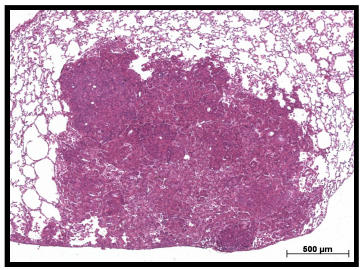
300 µm step-serial sectioning,
WHO classification

Bronchioalveolar

Adenoma

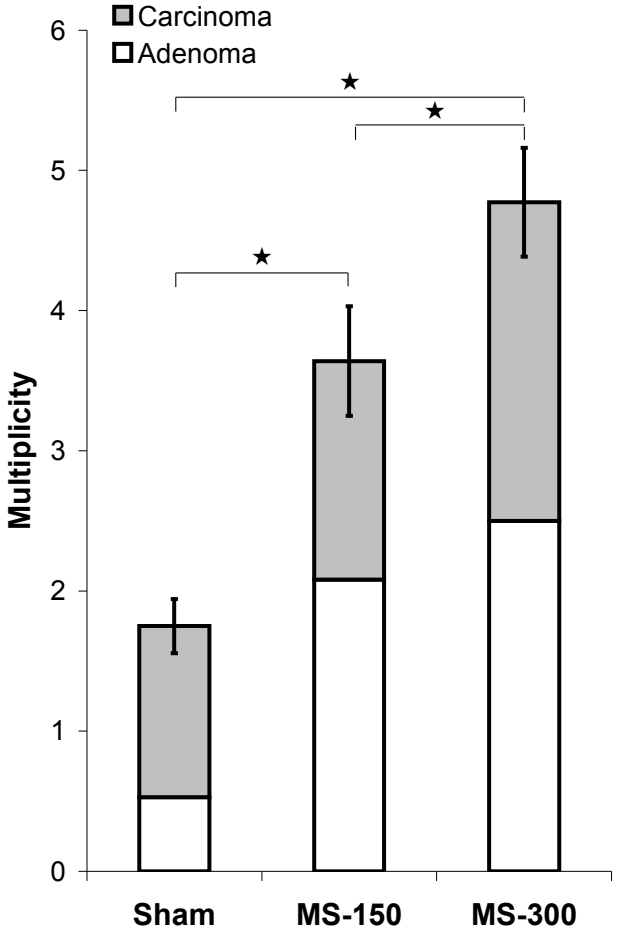


Adenocarcinoma



(N = 22 to 36; mean ± SE;
ANOVA followed by Tukey test)

Dose-Response Relationship after 18 Months of Inhalation



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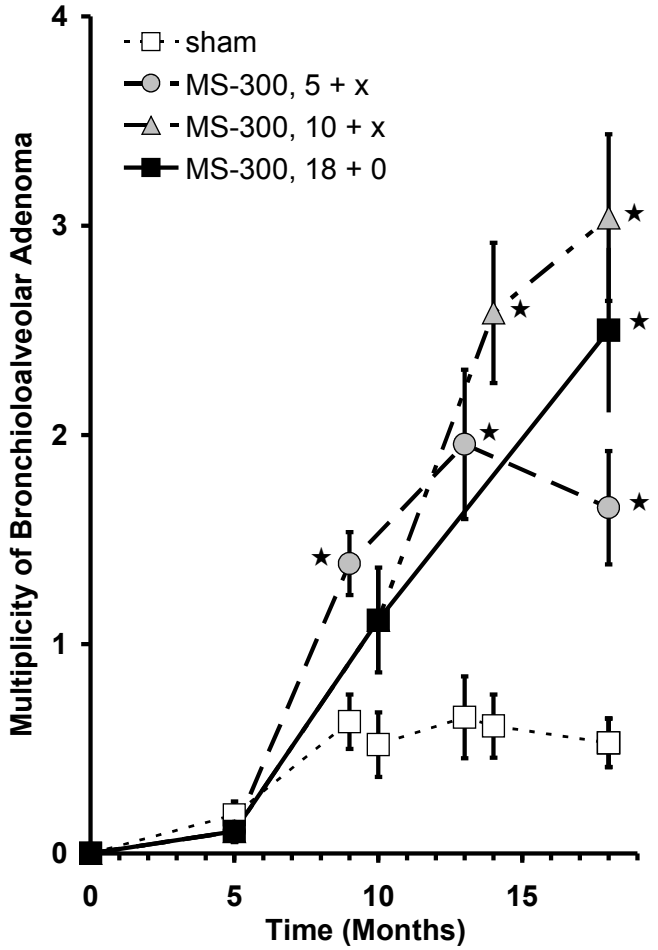
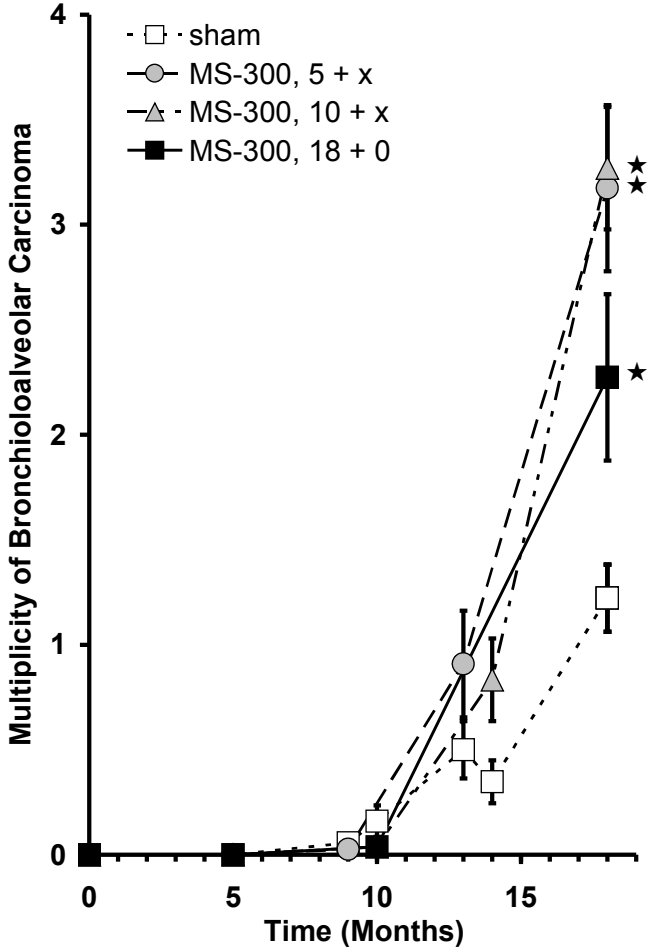
Time Course of Tumor Development

Adenoma Multiplicity



Adenocarcinoma Multiplicity



(N = 20 to 39; mean ± SE; ANOVA with sham, MS-150, and MS-300, followed by Tukey test)

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Transient Gene Induction by Smoke Exposure

Gene Symbol	Activity	Fold Change after Inhalation and Post-Inhalation Periods (months)						
		٠.٥ + ٠	٢.٥ + ٠	٥ + ٠	٥ + ٢ d	٥ + ٤	٥ + ١٣	١٨ + ٠

(MS-300 relative to time-matched sham controls; Agilent mouse genome oligo microarray (44K)

Groups of data columns 1 to 4: 4 lungs pooled for testing;
groups of data columns 5 to 7: 4 lungs tested individually;
Significance criteria: p<0.05, 2-fold change)

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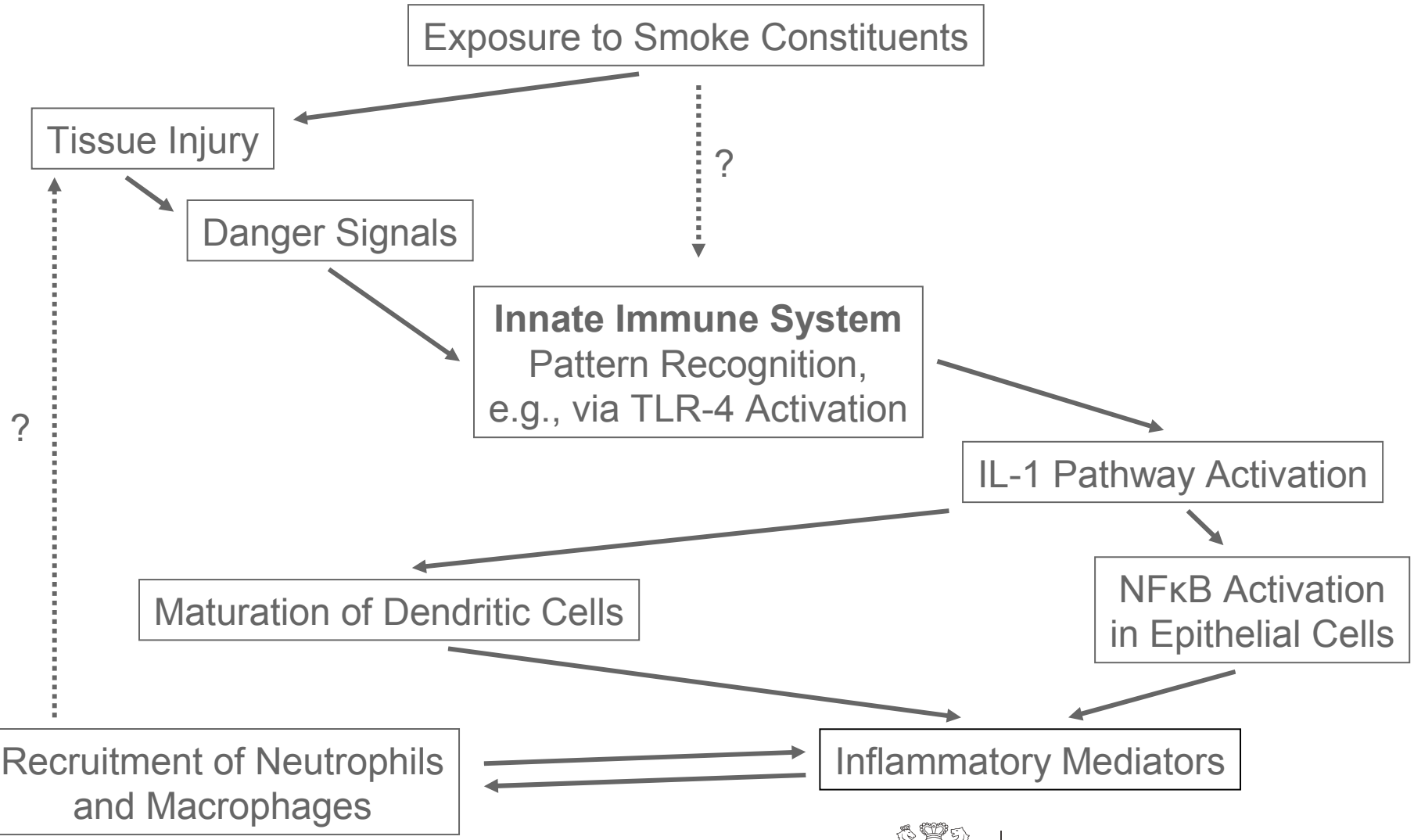
Scheme for Immunological Reactions to Smoke Inhalation, Step 1 (Possibly Reversible)



(according to Cosio et al., NEJM 2009)

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Gene Expression Changes Representative of an Innate Immune Response

Gene Symbol	Activity	Fold Change after Inhalation and Post-Inhalation Periods (months)						
		0.5 + 0	2.5 + 0	5 + 0	5 + 2 d	5 + 4	5 + 13	18 + 0
Innate Immune Response:								
il1r2	IL-1 receptor 2	4.6	4.0	4.4	1.5	2.0	3.6	20
il1rn	IL-1 rec. antag.	3.9	4.9	4.2	4.3	7.2	1.3	8.0
il1a	IL-1α	1.9	2.1	2.4	2.7	2.7	-1.1	2.9
il6	IL-6	2.5	3.2	1.8	1.8	-1.8	1.1	3.2
cxcl1	KC (IL-8)	7.8	9.1	5.6	6.2	3.8	2.5	9.6
ccl2	MCP-1	2.5	3.2	3.5	4.2	5.2	2.9	5.2
tnfa	TNF-α	1.7	1.7	1.6	1.6	2.0	2.2	2.9
csf2	GM-CSF	1.7	1.9	1.7	3.6	1.6	1.7	2.6

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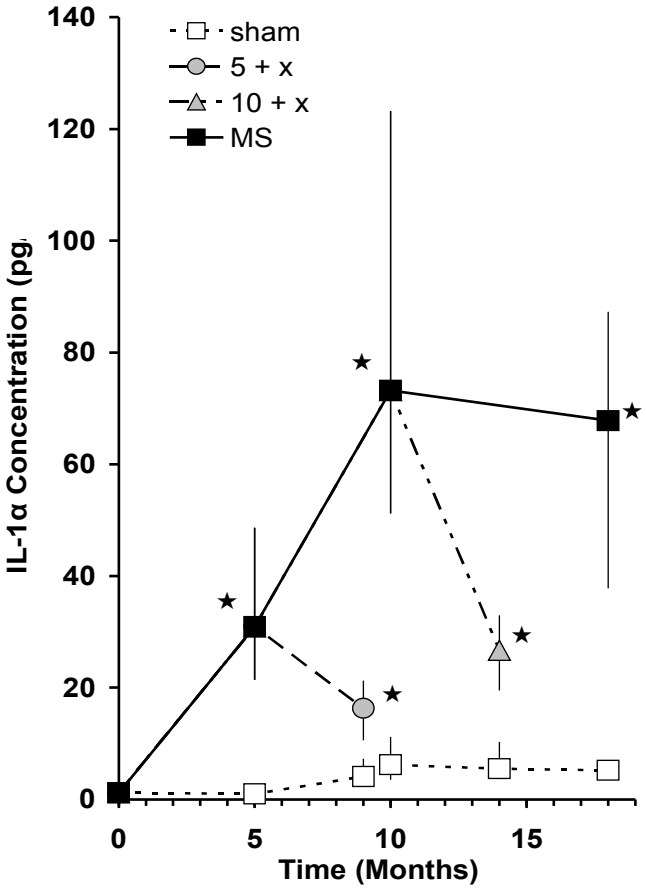
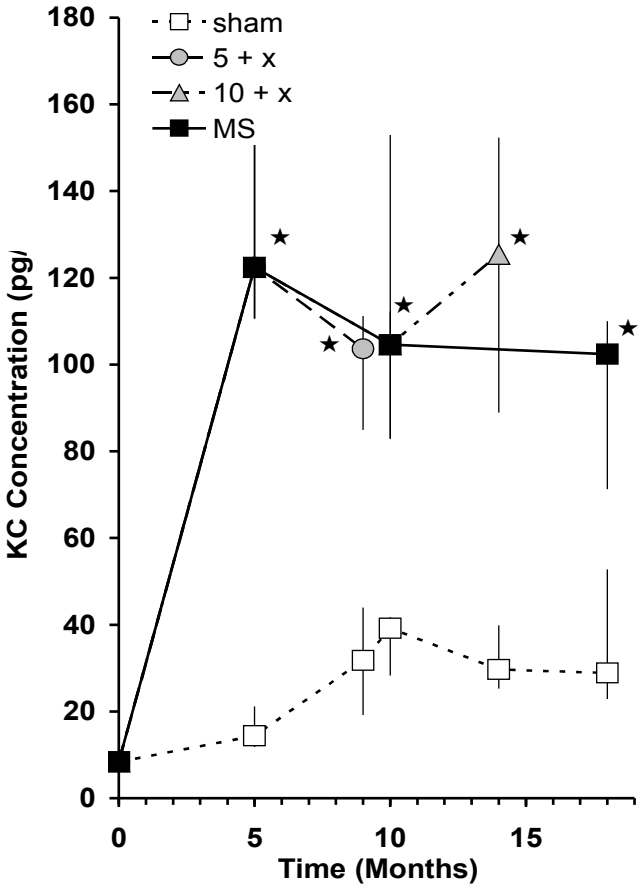
Early Immune Responses
(Assessed in Bronchoalveolar Lavage)

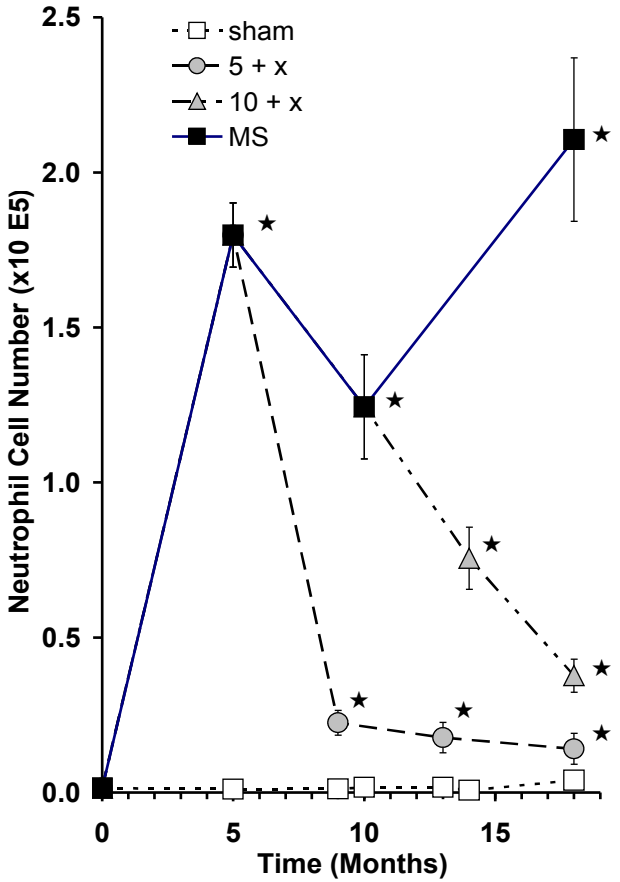
IL-1α Concentration



KC Concentration



Neutrophil Number



(N = 6 to 10; median and 25/75% quartiles for mediators, mean ± SE for cells; ANOVA with sham, MS-150, and MS-300, followed by Tukey test)

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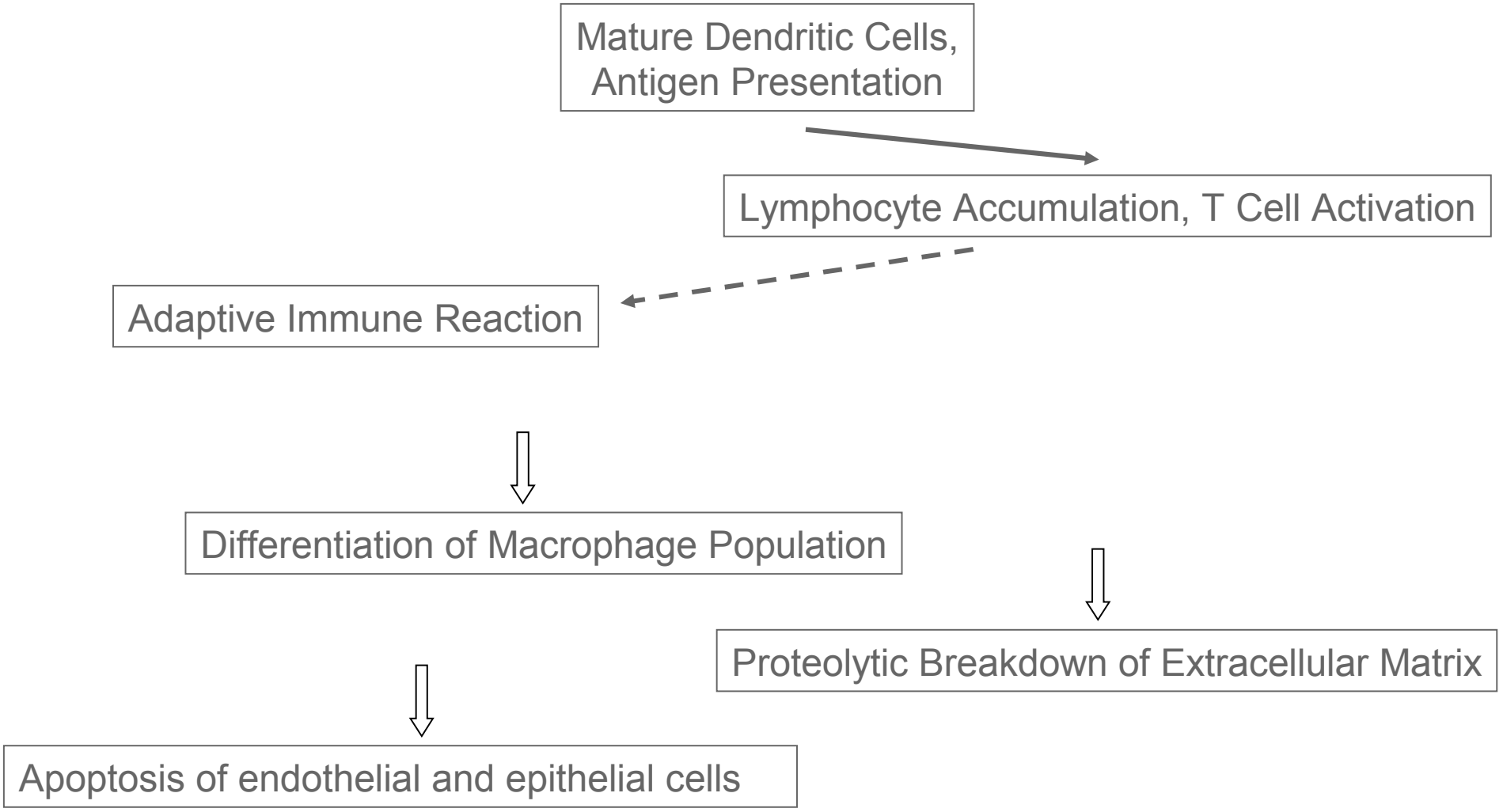
Scheme for Immunological Reactions to Smoke Inhalation, Steps 2 and 3 (Probably Irreversible)



(according to Cosio et al., NEJM 2009)

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Gene Expression Changes Representative of Dendritic Cell Maturation as well as T Cell and Macrophage Activation

Gene Symbol	Activity	Fold Change after Inhalation and Post-Inhalation Periods (months)						
		0.5 + 0	2.5 + 0	5 + 0	5 + 2 d	5 + 4	5 + 13	18 + 0
Dendritic Cell Markers:								
cd80	CD80	1.2	1.2	1.2	1.4	3.5	1.7	3.5
cd86	CD86	1.6	2.3	2.0	2.0	1.9	2.1	2.4
il12b	IL-12 p40	1.8	2.3	2.4	2.8	1.7	2.8	4.9
Ligands Involved in T Cell Activation:								
ccl20	MIP-3α	7.6	5.1	3.6	2.7	3.4	2.1	3.6
cxcl9	MIG	2.1	3.8	4.4	4.9	1.9	3.7	3.5
cxcl10	IP-10	2.3	1.9	3.0	5.3	1.4	2.2	2.5
Macrophage Activation Markers:								
cd68		2.8	4.7	5.1	4.0	3.3	1.8	4.8
msr1	scavenger rec.	2.8	6.6	7.0	3.9	3.9	1.6	7.7
npy	neuropeptide Y	1.9	6.3	5.3	6.4	55	2.9	23

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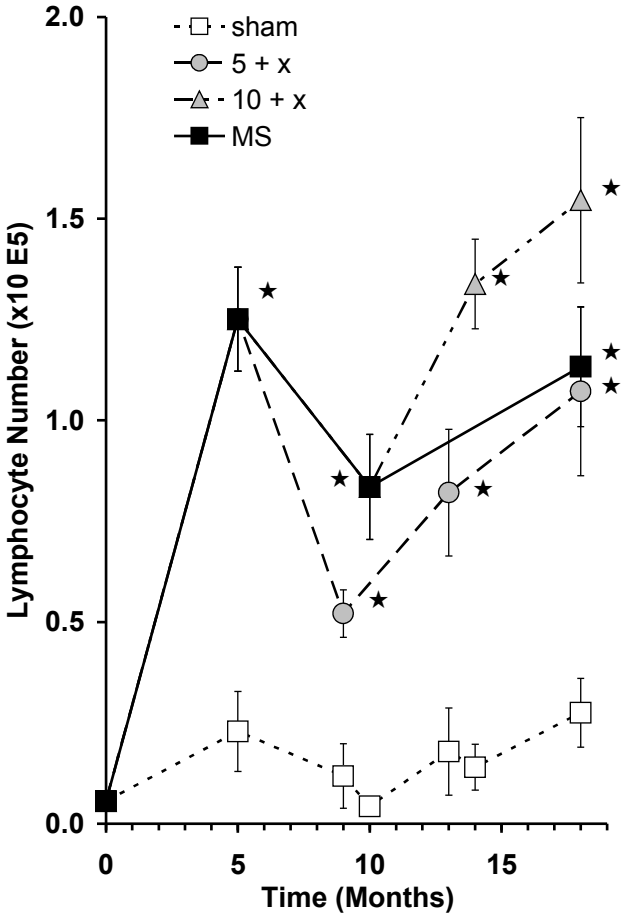
Lymphocyte Response

Total Lymphocyte Number in BAL



No change in lymphocyte differentiation
(collected from lung-associated lymph nodes)

(N = 6 to 10; mean ± SE for cells; ANOVA with sham, MS-150, and MS-300, followed by Tukey test)

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Gene Expression Changes Representative of Protease/Antiprotease Imbalance

Gene Symbol	Activity	Fold Change after Inhalation and Post-Inhalation Periods (months)						
		0.5 + 0	2.5 + 0	5 + 0	5 + 2 d	5 + 4	5 + 13	18 + 0
Proteinases:								
mmp12	metalloprot.	7.1	10	11	8.8	20	2.4	7.1
ctsk	cathepsin K	5.3	8.9	8.8	5.2	4.3	1.4	6.4
adam8	metallopeptid.	2.5	3.6	3.9	3.0	2.2	1.2	2.7
Proteinase Inhibitors:								
timp1		3.7	2.9	2.5	2.0	1.8	1.6	5.4
slpi		-1.7	1.4	-1.3	-1.5	2.6	2.8	5.7
serpina10	α_1-antiprotease	-1.1	1.1	2.5	1.6	2.2	2.8	5.8

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Concepts for the Role of Inflammation in COPD and Lung Cancer

- Common mechanisms (Yao and Rahman, 2009), e.g.:
  - Dysregulated inflammatory responses
  - Epithelial-to-mesenchymal transition
- Reduced incidence of lung cancer after anti-inflammatory corticosterone inhalation (Parimon et al., 2007)
- Relevance of emphysematous changes for tumorigenesis (Houghton et al., 2008):
  - Emphysema as a smoking-independent risk factor for lung cancer
  - Repair or maintenance activity as proliferative stimulus
  - Proteolytic activation of growth factors
    - Current study: increased expression of genes coding for, e.g., EGFR ligands (amphiregulin, epiregulin, betacellulin), insulin-like growth factor-1, and several fibroblast growth factors

Summary and Conclusions

- Successful development of a chronic mainstream smoke inhalation model for lung adenocarcinoma and emphysema in A/J mice
- Pronounced modulation of inflammatory processes:
  - Clear genotypic and phenotypic patterns of inflammation
  - Transient and sustained changes in gene expression and phenotypic markers upon cessation of smoke exposure
- Causal role of inflammatory effects in the chronic pathogenesis developing in this mouse model and their relevance to human smoking-related diseases remains to be established
- Current study concept in line with US National Heart, Lung, and Blood Institute workshop conclusions (Punturieri et al., 2009): *Lung cancer and COPD:*
  Needs and Opportunities for Integrated Research, e.g., use of animal models

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Thank you very much for your attention!

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