Cigarette Mainstream Smoke-Induced Lung Inflammation in A/J Mice

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Introduction

The A/J mouse has been described as a (mildly) susceptible animal model for cigarette smoke-induced emphysema [1] characterized by significant pulmonary inflammation and increased mean linear intercept.

Objective

Investigate inflammatory and histological changes in lungs from A/J mice following exposure to cigarette mainstream smoke as part of an overall effort to develop cigarette smoke-induced COPD animal models.

Study Design

- Female A/J mice, 6 months old at start of the study, 10/group (histopathology and lymph nodes) or 16/group (bronchoalveolar lavage)
- Exposure to 2R4F mainstream smoke or conditioned fresh air (sham), 5 d/wk, up to 5 months (after 2-week dose adaptation period):
  - 750 µg total particulate matter (TPM)/l for 2, 3, or 4 h/day; i.e., daily smoke dose of 1500, 2250, or 3000 µg TPM/(l * day)
  - Test atmosphere characterization

TPM =	735.0	± 43.0 µg/l
CO =	792.5	± 45.9 ppm
nicotine =	42.57	± 4.44 µg/l
formaldehyde =	0.48	± 0.05 µg/l
acetaldehyde =	49.10	± 2.69 µg/l
acrolein =	4.81	± 0.24 µg/l

- Necropsy at 3 and 5 months (1 d after last exposure)
  - Bronchoalveolar lavage (BAL) with 5 cycles of filling and emptying with 1 ml of PBS (1st cycle) or PBS + 0.325% BSA (2nd to 5th cycle)
  - Bronchial lymph node cells obtained by teasing tissue in HBSS + 5% FCS
  - 4% formalin instillation fixation and paraffin embedding of lungs; HE staining of 4 µm slices
- Statistics: analysis of variance (ANOVA) followed by Dunnett post-hoc test; statistical significance compared to sham: +, p <0.05; ++, p <0.01; +++, p <0.001; results are shown as mean ± SE or as median.

End Points

- Inflammatory mediators (cytokines and chemokines MMP-9 and TIMP-1) in BAL fluid (BALF) (Rodent Multi-Analyte Profile, Rules Based Medicine, Inc.) or ELISA (KC)
  - in cell-free supernatant out of 1st lavage cycle
- Cell differentiation in BALF [flow cytometry (FCM)]
- Activation marker expression in alveolar macrophages (FCM): CD86 (co-stimulatory molecule B7.2) and CD11b (Mac1 α-chain)
- Differentiation of lymphocytes in bronchial lymph nodes (FCM)
- Expression of activation markers on CD4 and CD8 cells in bronchial lymph nodes (FCM): CD44, CD62L, CD25, and CD69
- Histopathological evaluation of HE-stained lung slices

Abbreviations

GCP, granulocyte chemotactic peptide
GM-CSF, granulocyte-macrophage colony stimulating factor
IL, interleukin
KC, keratinocyte cytokine
LIF, leukemia inhibitory factor
MCP, macrophage chemotactic protein
M-CSF, macrophage colony stimulating factor
MDC, macrophage-derived chemoattractant
MFI, mean fluorescence intensity
MIP, macrophage inflammatory protein
RANTES, regulation upon activation, normal T-cell expressed, and secreted
TNF, tumor necrosis factor

References

[1] A. Guerassimov *et al.*, Am. J. Respir. Crit. Care Med. 170: 974 (2004)

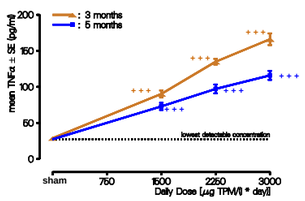
Results

Inflammatory Mediators in BALF

Inflammatory Cytokines

- Similar smoke effect seen for IL-1α, IL-2, IL-6, IL-7, IL-11, IL-17, IL-18, TNF-α.

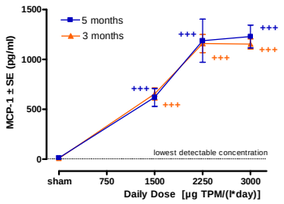
Example: TNF-α



Monocyte/Macrophage Chemoattractants

- Similar smoke effect seen for GM-CSF, LIF, M-CSF, MCP-1, MCP-3, MCP-5, MIP-1α, MIP-1β, MIP-1γ, RANTES.

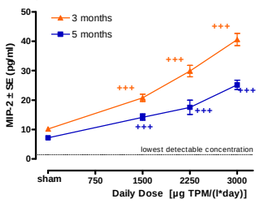
Example: MCP-1



Neutrophil Chemoattractants

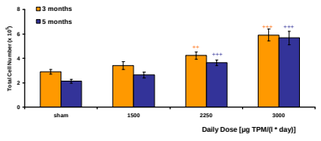
- Similar smoke effect seen for GCP-2, GM-CSF, KC, LIF, MDC, MIP-1α, MIP-1β, MIP-1γ, MIP-2.

Example: MIP-2

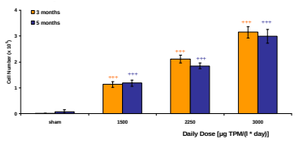


Inflammatory Cells in BALF

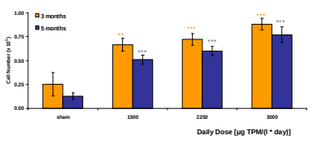
Cell Yield



Neutrophils



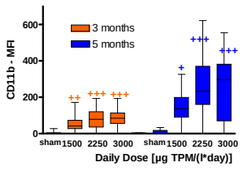
Lymphocytes



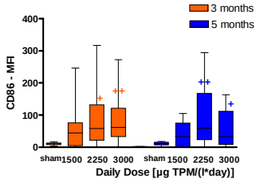
No increase in alveolar macrophages (data not shown).

Alveolar Macrophage Activation Marker Expression

CD11b (Mac1, α-chain)



CD86 (costimulatory molecule)

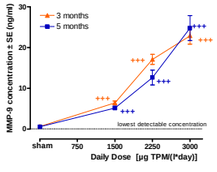


median; 1st Qu./3rd Qu.; min/max

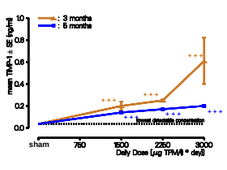
Difference between antibody fluorescence and isotype control fluorescence.

Proteases and Antiproteases in BALF

MMP-9



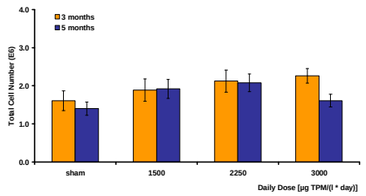
TIMP-1



Lymphocyte Differentiation in Bronchial Lymph Nodes

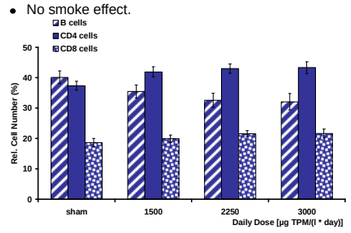
Cell Yield

- No smoke effect.



Lymphocyte Subpopulations (5 months)

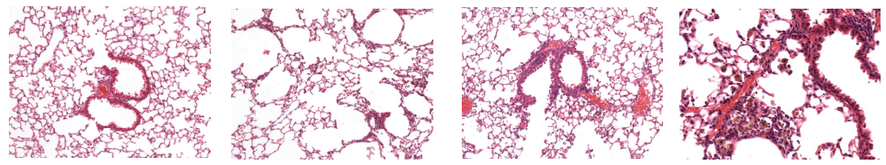
- No smoke effect.



No smoke effect seen for CD4 and CD8 lymphocyte expression of CD44 (hyaluronate receptor), CD62L (L-selectin), CD25 (IL-2 receptor type I), and CD69 (early activation antigen) (data not shown).

Histological Changes in Lungs

Sham Exposure **Smoke Exposure**



(5 months, 10x) [5 months, 1500 µg TPM/(l * day), 10x] [5 months, 3000 µg TPM/(l * day), 10x] [5 months, 3000 µg TPM/(l * day), 20x]

- Normal morphology of alveoli and bronchioli.
- Emphysematous destruction of alveolar septa.
- Peribronchiolar and perivascular infiltration by inflammatory cells.
- Alveoli contain brown-pigmented alveolar macrophages.

Summary and Conclusion

A/J mice exposed to cigarette mainstream smoke at daily doses of up to 3000 µg TPM/(l * day) showed the following:

- Pronounced pulmonary inflammation as indicated by increased concentrations of cytokines/chemokines and increased neutrophil and lymphocyte numbers in BALF
  - with no further increase between 3 and 5 months exposure.
- Macrophage activation as indicated by increased expression of CD11b and CD86.
- Indication of a protease-antiprotease imbalance as evidenced by a pronounced increase in MMP-9 compared to TIMP-1 in BALF. (MMP-9 activity not determined.)
- Emphysematous destruction and peribronchiolar and perivascular leukocyte infiltration as indicated by histopathological evaluation of the lung slices.
- No changes in bronchial lymph node lymphocytes.

The A/J mouse should be further investigated as a potential model for cigarette smoke-induced COPD.

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