Time-course of airway responsiveness and inflammatory mediator release in precision-cut lung slices from cigarette-smoke-exposed A/J mice

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Introduction

- Chronic obstructive pulmonary disease (COPD) is a disease characterised by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and worsens during the course of the disease (1) .
- Lungs in patients with COPD are chronically inflamed and elevated levels of pro-inflammatory mediators, released from structural or inflammatory cells, can be found in the sputum and bronchoalveolar lavage obtained from these patients (2).
- The chronic inflammatory response leads to structural changes and remodelling of the airways that has been shown to be accompanied by an increased sensitivity of airway smooth muscle to spasmogens such as methacholine (3, 4).

Aim

To study the effect of tobacco smoke exposure and subsequent smoking cessation on the function of intrapulmonary airways and inflammatory mediator release from mouse precision-cut lung slices (PCLS).

Materials and Methods

Animals

Female A/J mice (The Jackson Laboratory, USA); age at start of study ~ 12 weeks.

Exposure

Whole-body exposure to fresh, conditioned air (SHAM) or to cigarette mainstream smoke (MS) from the Reference Cigarette 3R4F at a concentration of 750µg total particulate matter (TPM)/l, for 4 hours per day, 5 days per week for 1 month, 5 months, or 5 months followed by a post-inhalation period of 2 months (5 + 2 months).

Preparation of precision-cut lung slices (PCLS)

- PCLS (approx. 250µm thick) were prepared using a Krumdieck Tissue Slicer. Lungs were prepared as described previously (5) with some modifications adapted to the mouse lung.
- PCLS were washed with buffer for 4 hours to remove agarose and debris from the airways and were kept in an incubator (37°C; 5% CO_2) until used in the experiment.

Cumulative methacholine concentration response curves

- Cumulative concentration response curves were generated from the following methacholine concentrations: 10^{-9}M, 10^{-8}M, $10^{-7.5}$M, 10^{-7}M, $10^{-6.5}$M, 10^{-6}M, $10^{-5.5}$M, 10^{-5}M, 10^{-4}M and EC_{50} values were calculated.

LPS stimulation of PCLS and mediator release

- PCLS (n=2/ well) were stimulated with 100ng/ml lipopolysaccharide (LPS) from *E.coli* for 24 hours.
- Matrix metalloproteinase 9 (MMP-9), tumour necrosis factor α (TNFα), interleukin-18 (IL-18), and the chemokines CCL3 (MIP 1α) and CXCL10 (IP-10) were measured using multiplex analysis (Aushon Biosystems, USA).

Results

Effect of chronic MS exposure on airway responsiveness

- 1 month: MS & SHAM: similar airway responsiveness and EC_{50} values.
- 5 months: MS: higher concentrations of methacholine needed to induce airway contraction. Maximum contraction increased compared to SHAM.
- 5 + 2 months: Comparable pattern of airway response 2 months after smoking cessation (Figure 1 and 2, Table 1).

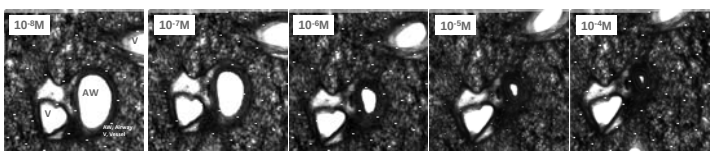

Figure 1. Methacholine-induced bronchoconstriction in mouse PCLS

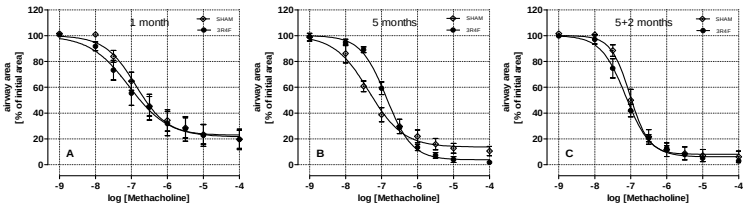

Figure 2. Concentration response curves to methacholine in mouse PCLS

● = 3R4F; ◊ = SHAM; mean ± SEM from n=5 animals

	1 month		5 months		5+2months	
	SHAM	3R4F	SHAM	3R4F	SHAM	3R4F
Top	100	100	100	100	100	100
Bottom	23±4	21±5	14±3	4±1	8±2	6±2
HillSlope	-1.0±0.2	-0.8±0.2	-0.9±0.1	-1.2±0.1	-1.6±0.05	-1.23±0.04
EC_{50}	1.3 x 10^{-7} M	8.6 x 10^{-8} M	4.4 x 10^{-8} M	1.3 x 10^{-7} M	9.5 x 10^{-8} M	7.2 x 10^{-8} M

Table 1 Airway contraction in response to methacholine maximum bronchoconstriction, HillSlope, and calculated EC_{50} values.

EC_{50} values were calculated by log-linear regression analysis (variable slope) within the upper limit (top) of 100 % and the maximum percent-reduction in airway area (bottom) measured at the highest concentration of methacholine. Data are shown as mean ± SEM.

Effect of chronic MS exposure on pro-inflammatory mediator release

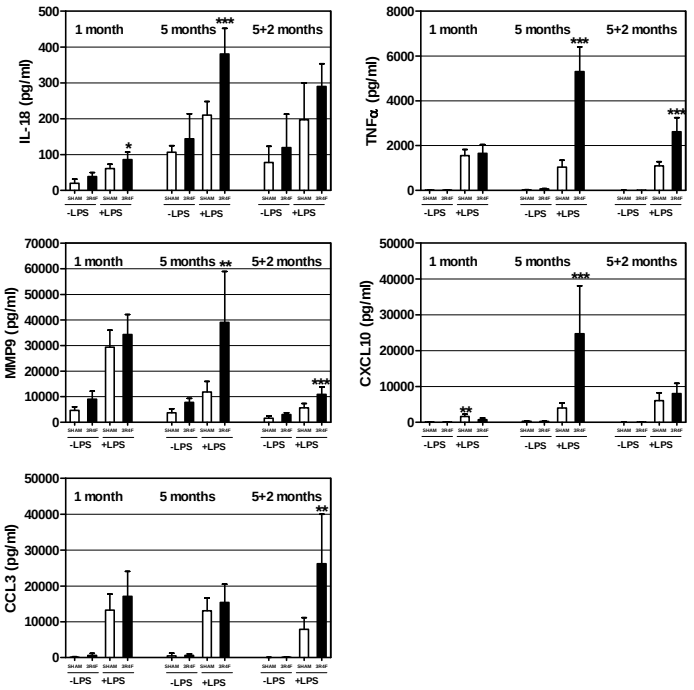

Figure 3. Pro-inflammatory mediator release from mouse PCLS under baseline conditions and in response to LPS.

Values are shown as mean ± SD from n=5 animals.* p<0.05; ** p<0.01; *** p<0.001 (SHAM vs. 3R4F)

Summary & Conclusions

- Chronic cigarette smoke exposure induces altered airway responsiveness and increased levels of inflammatory mediators are found in response to LPS in mice.
- Loss of bronchoalveolar attachments and emphysema formation as well as airway remodelling may be the underlying cause for this altered responsiveness.
- Since the pattern of airway contraction in PCLS from the SHAM and 3R4F groups are very similar 2 months after smoking cessation, we conclude that the underlying pathologies may be reversible in the mouse.
- Chronic cigarette smoke exposure leads to an exaggerated release of pro-inflammatory mediators in response to LPS and an elevated response remains even after smoking cessation.

References

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