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 characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles and gases. The chronic inflammatory response leads to structural changes in the airways (airway remodelling) that are often accompanied by an increased sensitivity of airway smooth muscle to non-specific spasmogens.

Aim: In this study we investigated the effect of tobacco smoke exposure on the function of intrapulmonary airways and inflammatory mediator release in mice.

Methods: Female A/J mice (n = 5 per time point per group) were exposed to conditioned fresh air (SHAM; 4 h/day) or to mainstream smoke from the Reference Cigarette 3R4F (4 h/day at 750 µg/l total particulate matter [TPM]) for 1 month, 5 months, or 5 months + 2 months post-inhalation. At the end of the exposure period, precision-cut lung slices (PCLS) were prepared. Cumulative concentration-response curves to methacholine (10^{-9} to 10^{-4} M) were generated and mediator release in response to overnight stimulation with 100 ng/ml lipopolysaccharide (LPS) was assessed for matrix metalloproteinase 9 (MMP-9), tumour necrosis factor α (TNFα), interleukin-18 (IL-18), and the chemokines CCL3 (macrophage inflammatory protein 1α) and CXCL10 (inducible protein-10).

Results: Airway responsiveness: A similar pattern of airway responsiveness with similar pEC_{50} values for methacholine was observed after 1 month (3R4F: 7.1 ± 0.1 vs. SHAM: 6.9 ± 0.1) as well as after 5 months inhalation + 2 months post-inhalation (3R4F: 7.1 ± 0.1 vs. SHAM: 6.9 ± 0.1). After 5 months, the pattern of the response to methacholine appeared to change in airways exposed to cigarette smoke. Significantly higher concentrations of methacholine were needed to induce bronchoconstriction.

Mediator release: After 1 month, mediator release from smoke-exposed and control PCLS in response to LPS was comparable. After 5 months, levels of MMP-9, IL-18, TNFα, and CXCL10 were significantly elevated in the supernatant of PCLS from smoke-exposed mice (SHAM vs. 3R4F; MMP-9: 11.8 ± 1.8 vs. 39.1 ± 8.9 ng/ml [$p<0.05$]; IL-18: 210 ± 17 vs. 381 ± 32 pg/ml [$p<0.01$]; TNFα: 1.0 ± 0.1 vs. 5.3 ± 0.5 ng/ml [$p<0.001$] CXCL10: 4.0 ± 0.5 vs. 24.7 ± 6 ng/ml [$p<0.001$]). Even after two months of smoking cessation, mediator release in response to LPS remained significantly elevated for TNFα, MMP-9, and CCL3 (SHAM vs. 3R4F; MMP-9: 5.6 ± 0.7 vs. 10.9 ± 1.2 ng/ml [$p<0.01$]; TNFα: 1.1 ± 0.08 vs. 2.6 ± 0.3 ng/ml [$p<0.001$]; CCL3: 7.8 ± 1.4 vs. 26 ± 6 ng/ml [$p<0.05$]).

Conclusion: Chronic cigarette smoke exposure induces altered airway responsiveness and increased inflammatory mediator release in response to LPS in mice. Precision-cut lung slices provide a suitable model with which to investigate these COPD-related changes