A mechanistic study of cigarette smoke-induced COPD in C57BL/6 mice: the impact of switching to pMRTP

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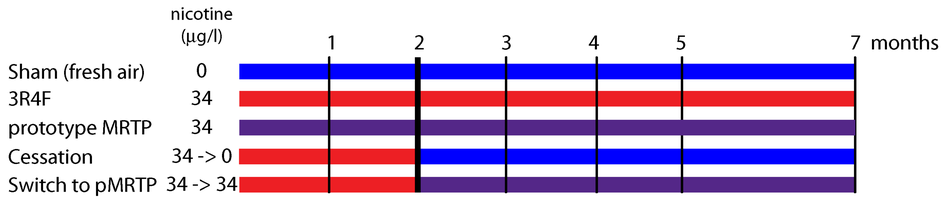
Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disorder characterized by a progressive, not fully reversible airflow obstruction, which is associated with an abnormal inflammatory response of the lungs to noxious particles or gases. The main risk factor for this disease is tobacco smoking, whose pathogenic action may be potentiated by other harmful agents such as air pollution and individual susceptibility. Suitable animal models play an important role in the understanding of the smoke-induced pathogenesis of COPD. It has been shown that the C57BL/6 mouse strain is a useful tool for the mechanistic investigation of this disease (Churg et al., 2008). Previously we have demonstrated that the chronic exposure of C57BL/6 mice to the aerosol generated by the reference cigarette 3R4F at a concentration of 750 µg/l total particulate matter (TPM) resulted in emphysematous changes as early as after 2 months. Development of the progression of the pathology and the impact of cessation (switching from 3R4F aerosol to filtered air) have been followed at the molecular, cellular and tissue level for up to 7 months of exposure (manuscript in preparation). This study assessed the impact of switching from the exposure to cigarette smoke from 3R4F to the aerosol generated by the prototypic modified risk tobacco product (pMRTP) or filtered air. To assess the development of emphysema we evaluated pulmonary inflammation and function, histopathological changes, and a multi-platform of molecular changes (transcriptomics, proteomics, and lipidomics) at months 1, 2, 3, 4, 5 and 7.

Study design and end points

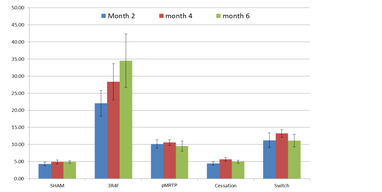


The mice were exposed to 3R4F (750 µg/l TPM), pMRTP (matched to the nicotine in 3R4F – 34.4 µg/l) or filtered air for 4 hours per day, 5 days per week, for up to 7 months. After 2 months of exposure to 3R4F, switching and cessation groups were exposed to pMRTP or filtered air, respectively. Animals were observed on a daily basis, body weight progression was monitored twice per week, exposure parameters (carboxy haemoglobin in blood and nicotine metabolites in urine) were measured 3 times during the study. Dissections were performed after 1, 2, 3, 4, 5,and 7 months of exposure. At each time point animals were allocated for the following end points: BALF (bronchoalveolar lavage fluid), for identification of infiltrated inflammatory cells in lungs and multi-analyte profiling; histopathological evaluation and morphometry of lungs; lung function and 'omics' (transcripomics and lipidomics).

Conclusions

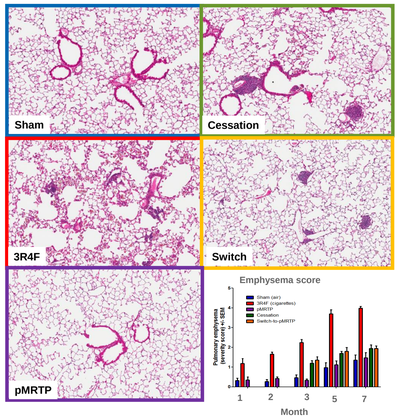
- The exposure to 3R4F cigarette smoke resulted in significant levels of pulmonary inflammation, declines in pulmonary function, and histopathological changes; these phenotypic changes were coherent with the molecular data;
- Chronic exposure to an aerosol from the pMRTP resulted in very little difference in all measured parameters when compared to the filtered air exposed animals;
- The biological response of switching to a pMRTP aerosol or filtered air following 2 months of 3R4F cigarette smoke exposure were very similar between the two conditions across the spectrum of endpoints assessed, and showed a generally positive effect;
- Differential gene expression associated with 3R4F exposure returned to a filtered air-like level following either switching to a pMRTP aerosol or filtered air;
- Histopathological assessment also showed a marked effect of switching, in which a partial or complete (depending on the inflammatory cell type) reversal of pulmonary inflammation was observed;
- These data collectively indicate a halting or regression of the disease genesis following switching.

Results



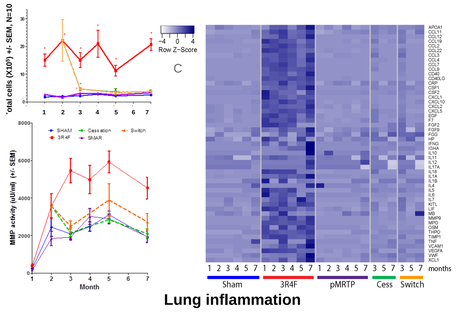
Carboxyhaemoglogin (HbCO) measurement in blood (%) - biomarker of exposure

After switching to aerosol from pMRTP or filtered air (cessation) levels of HbCO in blood of exposed animals are substantially lower compared to those found in blood of mice continuously exposed to 3R4F aerosol.



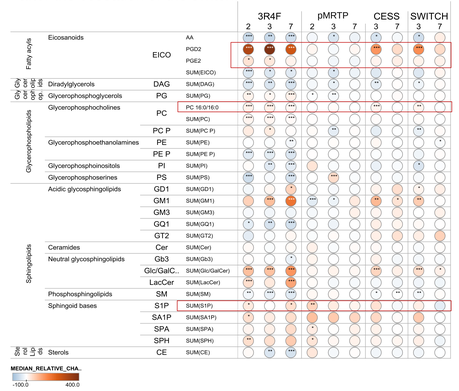
Histopathological evaluation of lungs

Degeneration of alveolar architecture as indicative for pulmonary emphysema was greater in animals continuously exposed to 3R4F than in those exposed to filtered air or pMRTP. In lungs of animals continuously exposed to 3R4F, a significant intra-alveolar accumulation of non-pigmented and brownish pigmented macrophages was seen which did not occur in animals exposed to an aerosol from the pMRTP. After switching to pMRTP or fresh air, a delayed interstitial accumulation of lymphocytis cells became evident. The level of emphysema was evaluated by the scoring of severity and by morphometrical measurements performed in a blinded manner by an external pathologist. After the switch or cessation, the evaluation revealed a certain level of reduction of severity compared to the emphysema seen in lungs from continuously exposed animals. However, even 5 months after the switch some tissue degradation was still evident.



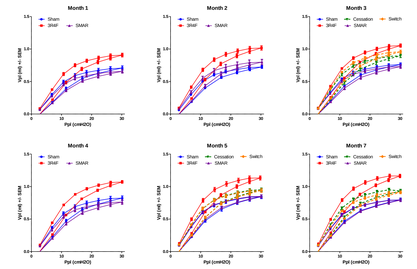
Lung inflammation

The absolute number of inflammatory cells in lung (A), the activity of matrix metalloproteinase (B) and the levels of inflammation-related chemokines detected in bronchoalveolar lavage fluid (C) were dramatically increased even after 1 month of exposure to 3R4F. Most of the inflammatory parameters measured reverted to the levels obtained with continuous exposure to filtered air or pMRTP-exposed animals after the cessation or the switch.



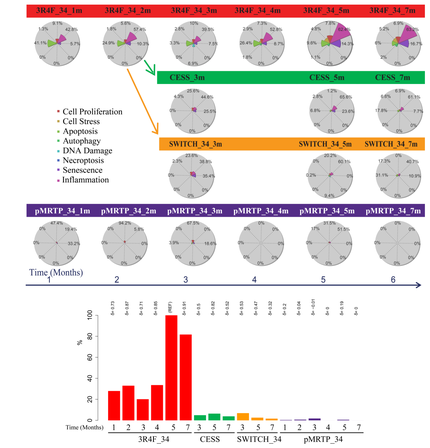
Changes in the lipid profile in the lungs

Exposure to 3R4F resulted in clearly distinguishable lung lipid profiles compared with sham exposure. These changes were alleviated by smoking cessation or switching to pMRTP within one month, and reached control levels at the 7-month time point.



Pulmonary function: pressure-volume (P-V) loops

Exposure of mice to 3R4F cigarette smoke resulted in a leftward shift of the P-V loops for both the inflation and deflation phase of the maneuver as compared to the results obtained with filtered air exposed animals. Measurements from pMRTP and filtered air exposed animals revealed very similar values during the course of the study, nearly overlapping at the 5th and 7th months. After the switch to the aerosol from pMRTP or cessation, P-V loops shifted right, but did not reach values obtained from animals continuously exposed to filtered air or pMRTP



Measurement of the perturbation of biological processes

Previously we have developed the method for quantitative measurement of the perturbation of biological processes (Hoeng et al, 2012; Martin et al, 2012). Relative biological impact factor (BIF) for lung tissue from 3R4F- and pMRTP-exposed vs. filtered air-exposed mice indicate strong perturbation of molecular networks caused by the exposure to 3R4F, while pMRTP had negligible effects on overall BIF. Transcriptional perturbations were dramatically reduced after the switch to pMRTP or filtered air. **Upper Panel**: Starplots showing the decomposition of the overall relative BIF into its main mechanistic components (from cell proliferation to inflammation) in the lung tissue for all treatment groups **Lower Panel**: The bar plot shows BIF values relative to the maximum response group (REF). The 'delta' values (−1 to 1) indicate how similar the underlying network perturbations are with respect to the REF.

References

Churg A, Cosio M, Wright JL (2008) Mechanism of cigarette smoke-induced COPD: insights from animal models. Am J Physiol Lung Cell Mol Physiol 294: L612-L631

Hoeng J, Deehan R, Pratt D, Martin F, Sewer A, Thomson TM, Drubin DA, Waters CA, de Graaf D, Peitsch MC (2012) A network-based approach to quantifying the impact of biologically active substances. Drug Discov Today 17: 413-418

Martin F, Thomson TM, Sewer A, Drubin DA, Mathis C, Weisensee D, Pratt D, Hoeng J, Peitsch MC (2012) Assessment of network perturbation amplitude by applying high-throughput data to causal biological networks. *BMC Syst Biol* **6:** 54

Gebel S, Lichtner RB, Frushour B, Schlage WK, Hoang V, Talikka M, Hengstermann A, Mathis C, Veljkovic E, Peck M, Peitsch MC, Deehan R, Hoeng J, Westra JW (2013) Construction of a Computable Network Model for DNA Damage, Autophagy, Cell Death, and Senescence Bioinformatics and Biology Insights 7: 97-117

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