Cigarette-Smoke-Induced Phenotype in Nrf2 KO Mice

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Introduction

Cigarette smoke (CS) is a complex chemical mixture estimated to be composed of up to 5000 different chemicals, including several class I carcinogens (according to IARC classification), a plethora of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and numerous free radicals. It is long-standing common scientific opinion that these compounds, which interact either directly or indirectly with target molecules in the O_2-containing extra-cellular and intra-cellular milieu of the respiratory tract or elsewhere in the organism, are among the drivers of CS-dependent chronic disease, mainly lung cancer, chronic obstructive pulmonary disease (COPD), and cardiovascular disease.

Research over the last decade has identified the transcription factor Nrf2 as being responsible for orchestrating cellular defense against any kind of oxidant stress on a large scale[1], though recent studies challenge this exclusive protective image by showing that the trans-activating potential of Nrf2 is abused by tumor cells, especially during lung tumorigenesis[2].

The pathway underlying the activation of Nrf2 is a major target of CS exposure, as shown in vivo by subjecting Nrf2 KO and Nrf2 WT mice to smoke inhalation over 5 months (3 different CS doses). Results show that CS-exposed Nrf2 KO mice, in contrast to their WT littermates, are strongly impaired regarding the expression of antioxidant and Phase II-related genes, although the effect appears to be compensated for to a minor extent by other transcription factors. Regarding the CS-induced phenotype in relation to the Nrf2 genotype, somewhat enhanced pathological effects were observed for CS-exposed Nrf2 KO mice in terms of a significant attenuation of body weight gain, increased scores for 'mean cord length', and several lung function parameters, which independently point to a compromised lung elasticity in CS-exposed Nrf2 KO mice.

This poster complements the oral presentation given by Thomas Mueller *From Cellular Genotype to Cigarette-Smoke-Induced Phenotype: The Case of Nrf2*.

Goal

Establish a model for CS-induced emphysema development and increase our understanding of the mechanisms underlying CS-induced emphysema development.

Approach

Analyze histopathology, morphometry, bronchoalveolar lavage (BAL) fluid measurements for free lung cells and cytokine levels, lung function parameters, µCT scanning, and gene expression in CS-exposed Nrf2 KO mice and their wild-type (WT) littermates.

Conclusions

Under the conditions of this study, disruption of the Nrf2 gene did not critically affect histopathological or morphometrical endpoints, free lung cells in BAL fluids, or cytokine levels in BAL fluids. However, several respiratory functional endpoints, as well as gene expression analysis (data not shown), suggest a greater impact of cigarette smoke on lung elasticity in the Nrf2 KO mice compared to WT mice.

Acknowledgements

We would like to thank Prof. M. Yamamoto for giving us permission to use the Nrf2 KO mouse. We would also like to thank the teams in Leuven and Cologne for performing the study.

Nrf2 knockout mouse (ICR background) (Reg No. 00984) was provided by RIKEN BRC which is participating in the National Bio-Resource Project of the MEXT, Japan.

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References

[1] Cho et al. *Antioxid. Redox Signal.* (2006) 8: 76-87

[2] Lau et al. *Pharmacol. Res.* (2008) 58: 262-270

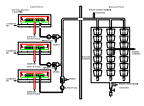
Exposure and Overall Status

Study Design

- Female Nrf2 KO mice and WT littermates
  - 12 weeks of age at exposure start
- Cigarette: 2R4F / mainstream smoke
- 4 study groups:
  - 1 sham group 3h
  - 3 smoke groups (750µg TPM/l) 2h, 3h, 4h
  - (30 mins fresh air after 1st hour, 60 mins fresh air after 2nd and 3rd hours)
- 5 days/week exposure for 5 months
- Adaptation period (750 µg from day 17)

Test Atmosphere	TPM Concentration (µg/l)	Daily Exposure (h)	Total Daily Concentration (µg/l)
Conditioned air (sham)		4	0
Smoke:			
LOW	750	2	1500
MEDIUM	750	3	2250
HIGH	750	4	3000

Study Groups

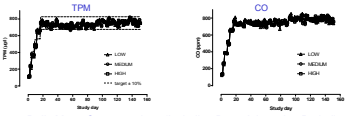


Smoke Exposure Setup

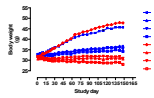
Group	Mean Formaldehyde Concentration	Mean Acetaldehyde Concentration	Mean Acrolein Concentration	Mean Nicotine Concentration
Short Name	(µg/l)	(µg/l)	(µg/l)	(µg/l)
SHAM				< DL (18)
LOW	0.58 ± 0.06 (19)	47.03 ± 2.39 (19)	4.85 ± 0.27 (19)	37.02 ± 4.04 (19)
MEDIUM and HIGH	0.53 ± 0.05 (18)	45.41 ± 1.73 (18)	4.66 ± 0.17 (18)	32.87 ± 3.97 (19)

(Comparable to historical data of 2R4F)

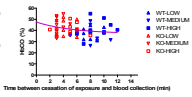
Test Atmosphere



Daily Mean Concentrations (Including Dose Adaptation Period)

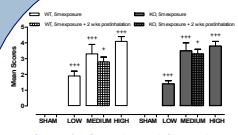


Body Weight Development

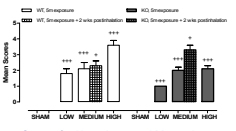


Blood HbCO Concentrations

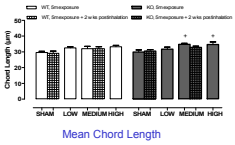
Histopathology and Morphometry



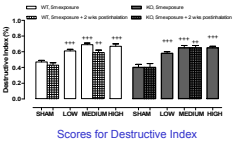
Scores for General Lung Inflammation



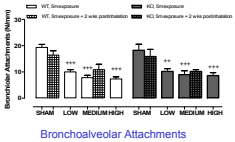
Scores for Non-pigmented Macrophages



Mean Chord Length



Scores for Destructive Index

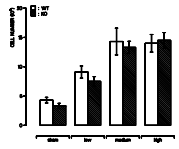


Bronchoalveolar Attachments

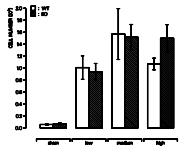
Summary Pathology

- Lung inflammation and alveolar emphysema was seen in both genotypes after 5 months of exposure to cigarette mainstream smoke.
- A dose-dependent increase in severity of inflammation as well as of alveolar emphysema was seen with increasing TPM concentration.

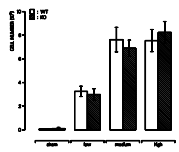
Free Lung Cells in BAL Fluid



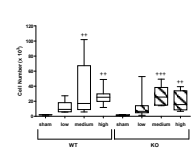
Total Free Lung Cells



Lymphocytes



Neutrophils

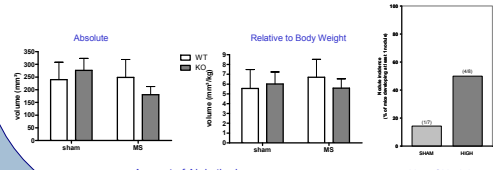


B-Cells

Summary Free Lung Cells in BAL Fluid

- A drastic increase in the absolute number of lymphocytes in BAL fluid was seen in both genotypes.
- The increase is not attributable to a single subpopulation, but is the result of an increase of approximately equal extent in each of the main lymphocyte subpopulations (CD4, CD8, and B cells; data not shown).
- The influx of these subpopulations was similar in Nrf2 WT and KO mice.

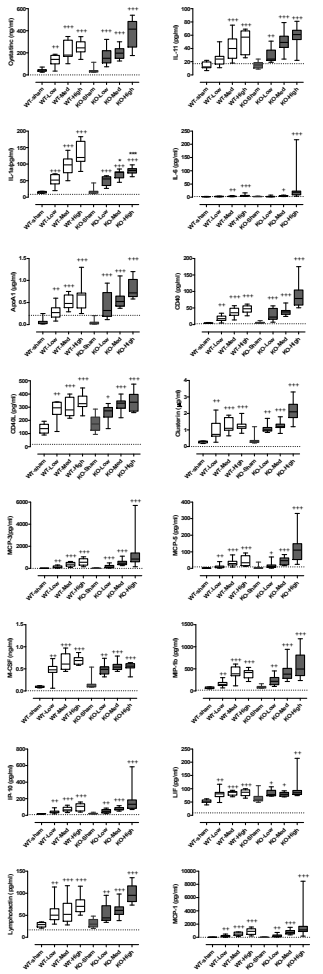
µCT Scanning (exploratory)



Amount of Air in the Lung

No. of Nodules

Multi-Analyte Profiling (MAP) in BAL Fluid



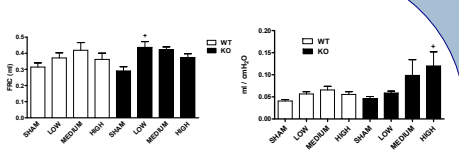
Summary MAP Analysis

- CD40, Clusterin, GMCSF, GSTM, IgA, IP10, MCP3, MCP5, MDC, MIP2, NGAL, OSM, TIMP1, TNFa, VCAM-1, VEGF, and VWF: slightly higher response for Nrf2 KO mice than WT mice.
- GCP2, IL-1 a, IL-1b, Insulin, MIP1g, Osteopontin and TPO: slightly lower response for Nrf2 KO mice than WT mice.
- Differences between Nrf2 KO and WT mice much less pronounced than difference between SHAM and smoke.

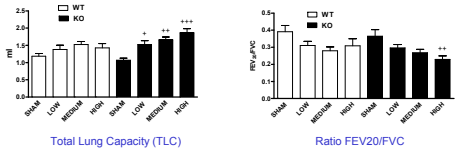
Statistics

+ = $p \leq 0.05$; ++ = $p \leq 0.01$; +++ = $p \leq 0.001$.
According to the endpoints measured, different statistical methods were applied.

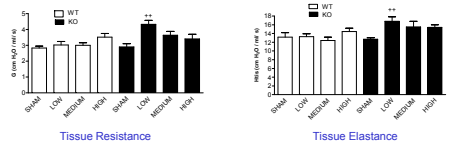
Lung Function Analysis



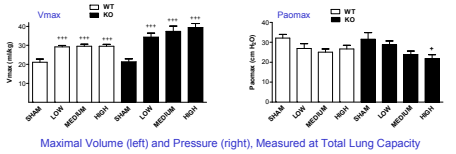
Functional Residual Capacity (FRC) | Compliance (at zero pressure, Cp0)



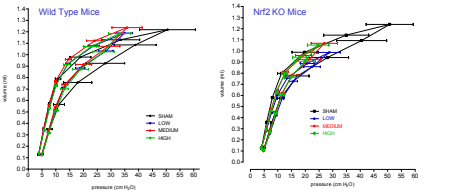
Total Lung Capacity (TLC) | Ratio FEV20/FVC



Tissue Resistance | Tissue Elastance



Maximal Volume (left) and Pressure (right), Measured at Total Lung Capacity



Quasi- Static Pressure Volume Loop

Summary Lung Function Analysis

- Strong indication of a reduction in lung elasticity, as seen by independent endpoints.
- More pronounced in Nrf2 KO mice than WT mice.

Gene Expression

Gene expression analysis in Nrf2 KO mice confirms the central role of Nrf2 in the cell's strategy to combat CS-induced damage and disclose new Nrf2 functions (data not shown). Gene expression discussed in detail in Thomas Mueller's presentation *From Cellular Genotype to Cigarette-Smoke-Induced Phenotype: The Case of Nrf2*