Inflammatory and Epithelial Changes in Lungs from Apolipoprotein-E-Deficient Mice after Chronic Cigarette Mainstream Smoke Exposure

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Introduction
Smoke (CS) exposure induces oxidative stress and inflammatory response in mice and humans alike. The biochemical alterations induce functional and morphologic changes thereby ultimately increasing the risk for the development of cancer, chronic obstructive pulmonary disease, and atherosclerosis.

Specifically, several studies in mice have shown that CS exposure leads to increased numbers of macrophages, neutrophils, and lymphocytes in bronchoalveolar lavage fluid (BALF) in mice. D’Hulst et al. reported a mixture of acute and chronic inflammatory changes in mice exposed to CS for 6 months. These changes are the result of a combination of innate and adaptive immune responses to CS; all augmented cell types are involved in both types of immune response.

As part of a study on the influence of cigarette mainstream smoke (MS) in combination with different diets on the development of atherosclerosis, we investigated the effects of MS on lungs of apolipoprotein-E-deficient mice (ApoE-/-). This mouse model develops atherosclerosis, a chronic inflammatory disease of large and medium arteries.

Objective
Investigate the inflammatory response and histopathological alterations in lungs from ApoE-/- mice exposed to MS and fed a normal chow or milk-fat-enriched diet.

Results

Body Weight Development
Body weight was up to 10% lower in the smoke-exposed groups compared to sham (normal chow diet) only.

![Body Weight Development Graph]

Carboxyhemoglobin
Carboxyhemoglobin levels were 11% ± 0.3 in the groups exposed to 100 µg TPM MS and 20% ± 0.6 in the groups exposed to 200 µg TPM MS.

Pigmented Macrophage Nests (PMN)
H&E-stained lung slice from an ApoE-/- mouse fed a milk-fat-enriched diet and exposed for 12 months to 200 µg TPM MS. Arrows indicate PMN.

Hypertrophy/Hyperplasia of the Alveolar Epithelium
H&E-stained lung slice from an ApoE-/- mouse fed a milk-fat-enriched diet and exposed for 12 months to 200 µg TPM MS. Arrows indicate hyperplasia of the alveolar epithelium associated with PMN.

Materials and Methods
Smoke Generation
CBF University of Kentucky Reference Cigarette, conditioning according to ISO standard 3492 (2007) and smoking parameters of a modified automated 30 puff smoking machine (type PARFO SM) equipped with a 4-puff Ballester pump according to ISO standard 20697.

Animals and Treatment
Mice were fed a milk-fat-enriched diet. 100 µg TPM/l MS showed a constant elevation in COHb levels compared to the sham group.

Histopathological Evaluation (12 mice/group)
- Embedding in paraplast, sectioning according to standard level (main bronchus)
- Staining with anti-neutrophil mAB-FITC (clone 7/4)
- Fixation of cells in 2% formalin
- Dissection and sample preparation after 3, 6, 9, or 12 months of exposure
- Carboxyhemoglobin (COHb)
- Collection of blood samples from tailcutaneous vessels of tail/ear-depressed mice during the last hour of exposure
- COHb determination using gas chromatography
- Bronchoalveolar Lavage (BAL) Cell Differentiation (5 mice/group)
- BAL: 3 hours after last exposure. 7 cycles with 5 min dry air and saline + 0.3% bovine serum albumin, filling at 15 cm water pressure, emptying at -8 cm water pressure
- Carboxyhemoglobin (COHb) levels were 11% ± 0.3 in the groups exposed to 100 µg TPM MS and 20% ± 0.6 in the groups exposed to 200 µg TPM MS. Arrows indicate PMN.

Incidence of Hypertrophy/Hyperplasia of Alveolar Epithelium and Incidence of PMN
The incidence of hypertrophy/hyperplasia and PMN in ApoE-/- mice exposed to 200 µg TPM MS increased from 20% (3 months) to 80% (12 months) in both diet groups. Hypertrophy/hyperplasia of the alveolar epithelium was seen only in single cells directly associated with the PMN.

Intestinal Lymphocytic Infiltrates
(A) Representative image of H&E-stained lung slice from an ApoE-/- mouse fed a normal chow diet and exposed for 12 months to 200 µg TPM MS. Arrows indicate intestinal lymphocytic infiltrates.
(B) Intestinal lymphocytic infiltrates were present in 80% of ApoE-/- mice exposed to 200 µg TPM MS in both diet groups only after 12 months of exposure.

Conclusion
These data indicate that chronic exposure of ApoE-/- mice to 200 µg TPM MS (but not to 100 µg TPM MS) resulted in non-neoplastic pathomorphological changes in the lung and an inflammatory response. The inflammatory response is a combination of innate and adaptive immune responses, as indicated by an increase in BAL neutrophils and lymphocytes.

Diet type had little effect. The inflammatory response was only marginally modulated by the milk-fat-enriched diet; histopathological alterations in the lung were not influenced by diet.

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References