Environ Health Perspect

DOI: 10.1289/EHP7255

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehp508@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

Supplemental Material

Repetitive Ozone Exposures and Evaluation of Pulmonary Inflammation and Remodeling in Diabetic Mouse Strains

James G. Wagner, Christina E. Barkauskas, Aaron Vose, Ryan P. Lewandowski, Jack R. Harkema, and Robert M. Tighe

Table of Contents

Table S13. Data for Figure 2C: Eosinophils (cells /ml x 10^4).

Table S14. Data for Figure 2D: Neutrophils (cells /ml x 10^4).

Table S15. Data for Figure 2E: Lymphocytes (cells /ml x 10^4).

Table S16. Data for Figure S3A: Neutrophils in Lung Tissue (%).

Table S17. Data for Figure S3B: Eosinophils in Lung Tissue (%).

Table S18. Data for Figure S3C: Macrophages in Lung Tissue (%).

Table S19. Data for Figure S3D: YM 1/2 Macrophages (% total macrophages).

Table S20. Data for Figure 3A: BALF IL-5 (pg/ml).

Table S21. Data for Figure 3B: BALF IL-13 (fg/ml).

Table S22. Data for Figure 3C: BALF IL-6 (pg/ml).

Table S23. Data for Figure 3D: BALF KC (pg/ml).

Table S24. Data for Figure 3E: BALF IL-17 (fg/ml).

Table S25. Data for Figure 3F: BALF IL-1β (fg/ml).

Table S26. Data for Figure 5G: Tissue Collagen (% lung parenchyma).

Table S27. Data for Figure 5I: Lung Hydroxyproline (μg/ml).

Table S28. Data for Figure 5H: Lung Hydroxyproline (μg/ml).

Table S29. Data for Figure 6A: Eotaxin mRNA.

Table S30. Data for Figure 6B: IL-13 mRNA.

Table S31. Data for Figure 6C: Mcp2 mRNA.

Table S32. Data for Figure 6D: Arg1 mRNA.

Table S33. Data for Figure 6E: Mmp12 mRNA.

Table S34. Data for Figure 6F: CCSP mRNA.

Table S35. Data for Figure 6G: Chia1 mRNA.

Table S36. Data for Figure 6H: Saa3 mRNA.

Table S37. Data for Figure S5G CCSP in Airway Epithelium (%).

Figure S1. *Body weights after air or ozone exposure for 13 days.* Final body weights of C57BL/6, KK and KKAy mice at the time of necropsy (A), and their change in body weight since the beginning of exposures to the time of necropsy 14 days later (B). C57BL/6J (open box), KK (gray box) and KKAy (black box). Data are expressed as mean ± SEM (n = 8/group). Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, c= significantly different from respective strain exposed to filtered air, $p < 0.05$. Summary data for panels A, and B can be found in Tables S2, and S3, respectively.

Figure S2. *Serum leptin and adiponectin in fasted mice after air or ozone exposure for 13 days.* Plasma concentrations of leptin (A) and adiponectin (B) were measured from fasted all mice at the time of necropsy, approximately 22 hours after the last O_3 exposure as described in Materials and Methods. C57BL/6J (open box), KK (gray box) and KKAy (black box). Data are expressed as mean ± SEM (n = 8/group). Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, c= significantly different from respective strain exposed to filtered air, $p < 0.05$. Summary data for panels A, and B can be found in Tables S7, and S8, respectively.

Figure S3. *Histologic assessment of ozone-induced pulmonary infiltration of neutrophils, eosinophils and macrophages in KKAy mice.* The density of neutrophils (A), eosinophils (B), total macrophages (C) and Ym1/2-positive macrophages (D) in lung tissue were immunohistochemically determined in lung tissue by high resolution morphometric methods as described in Materials and Methods. C57BL/6J (open box), KK (gray box) and KKAy (black box). (E) Representative image of lung tissue from KKAy mouse that was stained with major basic protein to identify eosinophils (arrows) in the centriacinar lesions (identified with asterisk). tb = terminal bronchial, ad = alveolar duct, and a = alveoli. Data are expressed as mean ± SEM (n = 8/group). Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, c= significantly different from respective strain exposed to filtered air, $p < 0.05$. ND = not detected. Summary data for panels A, B, C, and D can be found in Tables S16, S17, S18, S19, respectively.

Figure S4. *α-Smooth Muscle Actin Staining in KKAy mice.* Tissue sections from air-exposed (A) and repetitive O_3-exposed (B) KKAy mice underwent staining for α-smooth muscle actin (SMA) to identify myofibroblasts. α-SMA staining in air-exposed animals is noted in the subendothelial space (dashed arrow), while in the O_3-exposed mice α-SMA staining is noted in the centriacinar regions (solid arrow). Images are representative images. a = alveoli, ad = alveolar duct, e = endothelium.

Figure S5. *Immunofluorescense staining of SFTPC, CCSP and HA in KK and KKAy mice.* Increased sized images of KK and KKAy strains for visualization of the differences in staining and morphology in the air- and O_3-exposed KK and KKAy strains.

Figure S6. *CCSP immunohistology staining and morphometry in C57BL/6J, KK and KKAy mice.* Light photomicrographs of a centriacinar region in the lungs of C57BL/6 mice (A, B), KK mice (C, D) and KKAy mice (E, F) exposed to air (A, C, E) or ozone (B, D, F). Tissues were immunohistochemically stained for Club Cell Secretory Protein (CCSP; solid arrow; red chromagen) in epithelial cells (e) lining the terminal bronchiole (TB). Area of alveolitis (marked with stippled arrow), which included alveolar septal thickening, type two alveolar epithelial hyperplasia and macrophage accumulation in alveolar airspaces observed in the proximal alveolar duct (AD) and adjacent alveolar parenchyma (a). (G) Morphometry quantification of the airway was performed for CCSP in air- and O_3- exposed C57BL/6, KK and KKAy strains. Data were analyzed using a completely randomized analysis of variance with factors of mouse strain and exposure, and comparisons of group means made with the Student–Newman–Keuls *post hoc* test. a=significantly different from similarly exposed C57BL/6 mice, b= significantly different from similarly exposed KK mice, $p < 0.05$. Summary data for panel G can be found in Table S37.