

Title of Dataset

Inconsequential Role for Chemerin-Like Receptor 1 in the Manifestation of Ozone-Induced Lung Pathophysiology in Mice_Dataset

Materials and Methods

Animals

Four breeding pairs of mice heterozygous for a null mutation in the gene encoding chemerin chemokine-like receptor 1 (*Cmklr1*) were purchased from Deltagen, Inc. (San Mateo, CA) via Charles River Laboratories (Wilmington, MA). These mice were subsequently bred to generate male and female mice homozygous for a null mutation in the gene encoding *Cmklr1* [chemerin-like receptor 1 (CMKLR1)-deficient mice]. Male mice were used exclusively in this study, and since CMKLR1-deficient mice were backcrossed into a C57BL/6NCrl genetic background for at least five generations, male C57BL/6NCrl mice were purchased from Charles River Laboratories and used as wild-type controls. All mice were at least twelve weeks of age when subjected to any experimental procedures. Mice were housed as previously described (1), and the care and use of all animals in this study adhered to the guidelines of the National Institutes of Health (Bethesda, MD). Finally, each of the experimental protocols used in this study was approved by the Animal Welfare Committee of The University of Texas Health Science Center at Houston (Houston, TX).

Filtered Room Air (Air) or Ozone (O₃) Exposure

Each mouse was removed from its micro-isolator cage, weighed, and placed in its own cell that was part of a larger stainless steel wire mesh cage, which was then positioned inside a powder-coated aluminum and Plexiglas® exposure chamber. Without access to food or water, mice were subsequently exposed to either air or O₃ (2 parts/million) for three hours. After the exposure ceased, mice were returned to their micro-isolator cage and had access to food and water *ad libitum* until weighed again immediately before euthanasia or anesthesia either four- or twenty-four-hours following cessation of exposure.

Blood Collection, Bronchoalveolar Lavage (BAL), and Lung Harvest

Mice were euthanized with an intraperitoneal injection of pentobarbital sodium (200 mg/kg; Vortech Pharmaceuticals, Ltd., Dearborn, MI) four- or twenty-four-hours following cessation of exposure to either air or O₃. Once the animal failed to elicit ocular and pedal withdrawal reflexes, a median thoracotomy was performed, and blood was collected from the right ventricle of the heart. An aliquot of whole blood was diluted in Turk blood diluting fluid (Ricca Chemical Company®; Arlington, TX), and the total number of blood leukocytes enumerated with a hemacytometer (Hausser Scientific; Horsham, PA). Serum was subsequently isolated from blood and stored at -20°C until needed. After blood was collected, the lungs were lavaged a total of four times with ice-cold lavage buffer [1× phosphate-buffered saline (PBS) containing 0.6 mM ethylenediaminetetraacetic acid]. The lavagates were pooled, the liquid and cellular components of the lavagate separated by centrifugation, and the supernatant stored at -80°C until needed. The cell pellet that remained after centrifugation was resuspended in 1 mL of Hanks' balanced salt solution (HyClone Laboratories, Logan, UT), and the total number of cells in this suspension enumerated with a hemacytometer. For differential cell analysis, BAL cells were first deposited onto microscope slides using a CytoZEN cytology centrifuge (Hettich Instruments; Beverly, MA). Subsequently, the slides were air-dried and stained with the Hema 3 stain set (Fisher Diagnostics; Middletown, VA). The cells that were deposited on to the slides were examined under a light microscope at a total magnification of 400× for differential counts. Finally, when lavages were complete, the animal's circulation was flushed with 10 mL of ice-cold 1× PBS, the left main bronchus severed, and the left lung removed from the animal and snap frozen in liquid nitrogen and stored at -80°C until needed for ribonucleic acid (RNA) extraction.

RNA Extraction, Complementary Deoxyribonucleic Acid (cDNA) Synthesis, and Reverse Transcription-Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR)

Using previously described methods (1, 2), total RNA was extracted from frozen lung tissue, complementary cDNA synthesized from messenger RNA (mRNA), qPCR performed, and data analyzed using the comparative threshold cycle method so that the relative abundance of *Cmklr1* mRNA four- or twenty-four-hours following cessation of exposure to O₃ was expressed relative to the abundance of *Cmklr1* mRNA following cessation of

exposure to air. All data were normalized to the abundance of glucuronidase, beta (*Gusb*) mRNA, a reference gene. Primers for *Cmklr1* and *Gusb* were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA).

Cytokine and Protein Quantification

BAL supernatant and/or serum adiponectin, chemerin, chitinase-3-like protein 1, hyaluronan, interleukin (IL)-6, IL-11, keratinocyte chemoattractant, macrophage inflammatory protein (MIP)-2, MIP-3 α , osteopontin, and receptor for advanced glycation end-product were quantified in BAL supernatant using Quantikine™ or DuoSet® enzyme-linked immunosorbent assays from R&D Systems, Inc. (Minneapolis, MN) while BAL albumin and protein were quantified, respectively, with an enzyme-linked immunosorbent assay (Immunology Consultants Laboratory, Inc.; Portland, OR) or a Pierce™ Bicinchoninic Acid (BCA) Protein Assay Kit from Thermo Fisher Scientific Inc. (Waltham, MA). If a serum or BAL supernatant analyte was below the minimum detectable concentration of an immunoassay, we assigned the sample a value, which was calculated by dividing the minimum detectable concentration of the analyte by the square root of two.

Quasi-Static Respiratory System Pressure-Volume (PV) Curves and Airway Responsiveness to Acetyl- β -Methylcholine Chloride

Twenty-four hours following cessation of exposure to either air or O₃, mice were (1) anesthetized with pentobarbital sodium (50 mg/kg; Oak Pharmaceuticals, Inc.; Lake Forest, IL) and xylazine hydrochloride (7 mg/ml; Vedco Inc.; Saint Joseph, MO), (2) tracheostomized with an 18-gauge tubing adaptor (Becton, Dickinson and Company; Franklin Lakes, NJ), and (3) ventilated at a frequency of 2.5 Hz, a tidal volume of 0.3 mL, and a positive end-expiratory pressure of 3 cm H₂O for the generation of quasi-static respiratory system PV curves and the measurement of airway responsiveness to acetyl- β -methylcholine chloride (Sigma-Aldrich, Inc.; St. Louis, MO) as we previously described (2, 3). From each PV curve, quasi-static respiratory system elastance (E_{stat}) was calculated. After the generation of PV curves was complete, respiratory system impedance (Z_{RS}) was determined using the forced oscillation technique as we previously described (3). The constant-phase model was used to partition Z_{RS} into components representing airway resistance (R_{aw}), the coefficient of lung tissue damping (G), and the coefficient of lung tissue elastance (H) following administration of 1 \times PBS (Sigma-Aldrich, Inc.) alone and following administration of increasing concentrations of acetyl- β -methylcholine chloride dissolved in 1 \times PBS (0.1 mg/ml – 100 mg/ml). The *flexiVent* (SCIREQ Scientific Respiratory Equipment; Montréal, Québec, Canada) was used to ventilate the lungs, deliver stepwise inspiratory and expiratory volume increments for the generation of PV curves, and superimpose sinusoidal forcing functions of multiple frequencies on the breathing frequency of the animal (2.5 Hz) for the determination of Z_{RS} .

Lung Mass and Lung Wet-to-Dry Weight Ratio. Mice were weighed and then euthanized with an intraperitoneal injection of sodium pentobarbital twenty-four-hours following cessation of exposure to air. Subsequently, each mouse was subjected to a thoracotomy, which was followed by excision of the right and left lung lobes. After removing extraneous tissue from the lung lobes, the lobes were conjointly weighed on an analytical balance immediately after excision, and their mass was designated as the wet weight. Afterwards, the lobes were promptly placed in an oven for five days at 65°C. When five days had passed, the lobes were removed from the oven and conjointly weighed again on the same analytical balance. This mass was designated as the dry weight. The wet-to-dry weight ratio for the lungs was determined by dividing the wet weight *via* the dry weight.

References

1. **Razvi SS, Richards JB, Malik F, Cromar KR, Price RE, Bell CS, Weng T, Atkins CL, Spencer CY, Cockerill KJ, Alexander AL, Blackburn MR, Alcorn JL, Haque IU, and Johnston RA.** Resistin deficiency in mice has no effect on pulmonary responses induced by acute ozone exposure. *Am J Physiol Lung Cell Mol Physiol* 309: L1174-1185, 2015.
2. **Malik F, Cromar KR, Atkins CL, Price RE, Jackson WT, Siddiqui SR, Spencer CY, Mitchell NC, Haque IU, and Johnston RA.** Chemokine (C-C Motif) Receptor-Like 2 is not essential for lung injury, lung inflammation, or airway hyperresponsiveness induced by acute exposure to ozone. *Physiol Rep* 5: 2017.
3. **Barreno RX, Richards JB, Schneider DJ, Cromar KR, Nadas AJ, Hernandez CB, Hallberg LM, Price RE, Hashmi SS, Blackburn MR, Haque IU, and Johnston RA.** Endogenous osteopontin promotes ozone-induced neutrophil recruitment to the lungs and airway hyperresponsiveness to methacholine. *Am J Physiol Lung Cell Mol Physiol* 305: L118-129, 2013.

