Title of Dataset

Interleukin-11 Receptor Subunit Alpha-1 is Required for Maximal Airway Responsiveness to Methacholine After Acute Exposure to Ozone

Materials and Methods

Animals

Mice heterozygous for a null mutation in the gene encoding interleukin 11 receptor, alpha chain 1 (*Il11ra1*; IL-11Rα1$^{+/-}$ mice) were purchased from The Jackson Laboratory (Bar Harbor, ME). These mice were subsequently bred to generate male mice homozygous for a null mutation in the gene encoding *Il11ra1* (IL-11Rα1-deficient mice) and female IL-11Rα1$^{+/-}$ mice. Because female IL-11Rα1-deficient mice are infertile, male IL-11Rα1-deficient mice and female IL-11Rα1$^{+/-}$ mice were bred to generate male IL-11Rα1-deficient mice for our study. Male wild-type C57BL/6J mice were purchased from The Jackson Laboratory and used as wild-type controls since IL-11Rα1$^{+/-}$ mice were backcrossed into a C57BL/6J genetic background for twelve generations. 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 (O_3) 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 O_3 (2 ppm) for three hours. After the exposure ceased, mice were returned to the micro-isolator cage they occupied prior to the exposure until either four- or twenty-four-hours following cessation of exposure. Mice were weighed again immediately before euthanasia or anesthesia.

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 air or O_3. 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. 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 (Hausser Scientific; Horsham, PA). 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 *Il11ra1* mRNA four- or twenty-four-hours following cessation of exposure to O_3 was expressed relative to the abundance of *Il11ra1* mRNA following cessation of exposure to air. All data were normalized to the abundance of hypoxanthine guanine phosphoribosyl transferase

(*Hprt*) mRNA, a reference gene. Primers for *Il11ra1* and *Hprt* were purchased from Qiagen (Germantown, MD) and Bio-Rad Laboratories, Inc. (Hercules, CA), respectively.

Cytokine and Protein Quantification

BAL supernatant and/or serum IL-11, adiponectin, IL-6, KC, MIP-3α, hyaluronan, osteopontin, and soluble tumor necrosis factor receptor (sTNFR) 1 and 2 were quantified by either Quantikine™ or DuoSet® ELISA kits according to the manufacturer's instructions (R&D Systems, Inc.; Minneapolis, MN). Total BAL protein was quantified using the Bio-Rad protein assay (Bio-Rad Laboratories, Inc.) also according to the manufacturer's instructions.

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

Twenty-four hours following cessation of exposure to either air or ozone, 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 H_2O for the generation of quasi-static respiratory system PV curves and the measurement of airway responsiveness to methacholine (Sigma-Aldrich, Inc.; St. Louis, MO) as we previously described (2, 3). From each PV curve, A, an estimate of inspiratory capacity; K, curvature of the upper portion of the expiratory limb of the PV curve; C_{stat}, quasi-static respiratory system compliance; and Area, respiratory system hysteresis, were calculated. A, K, and C_{stat} were determined using the Salazar–Knowles equation (4). 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× PBS (Sigma-Aldrich, Inc.) alone and following administration of increasing concentrations of methacholine dissolved in 1× 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}.

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.
4. **Salazar E and Knowles JH**. An analysis of pressure-volume characteristics of the lungs. *J Appl Physiol* 19: 97-104, 1964.