SUPPLEMENTARY MATERIAL

Respiratory syncytial virus Preparation

Respiratory syncytial virus long strain was grown in HEp-2 cells (American Type Culture Collection, Manassas, VA, USA) and purified by polyethylene glycol precipitation, followed by centrifugation on 35–65% discontinuous sucrose gradients. The virus titer of the purified RSV was determined by a methylcellulose plaque assay and ranged from 8–9 log10 plaque forming units (PFU)/ml. Virus pools are aliquoted, quick-frozen in liquid nitrogen, and stored at −70°C until used. Viral preparations are routinely tested for LPS and cytokine contamination.

CS Exposure and Infection of Mice

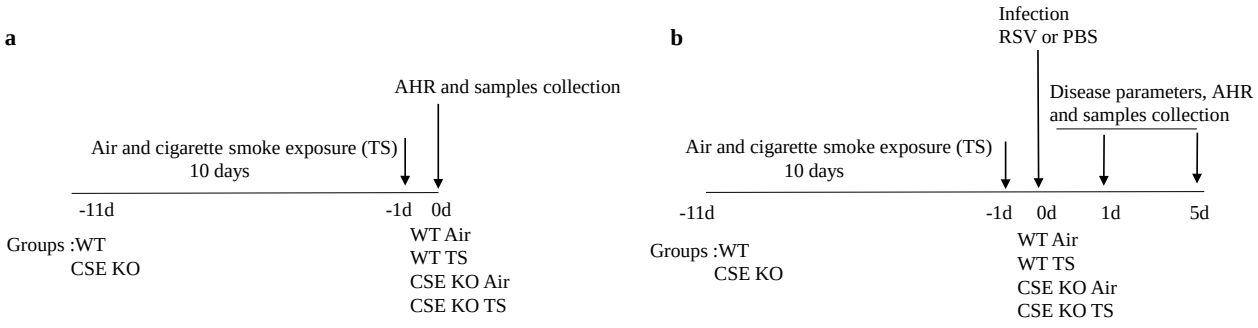
Cystathionine γ-lyase (CSE) deficient mice (CSE KO) were used to examine the role of endogenous H_2S in the pathogenesis of TS exposure. 10-12 weeks male and female C57BL/6J mice (wild type, WT) used in this work were purchased from The Jackson Laboratory (Bar Harbor, Maine). CSE KO mice on C57BL/6J background were generously provided by Dr. Solomon Snyder, Johns Hopkins University, Baltimore, MD. Both male and female CSE KO and WT age-matched mice were used in the experiments. Groups of CSE -/- (KO) and wild type control (WT) mice were subjected to whole body exposure of either TS (as side-stream, TS) or filtered air (5 h/day, 5 days/week, for two weeks). The Standard Reference Cigarettes 3R4F used in this study were obtained from the Tobacco and Health Institute at the University of Kentucky (http:// www.ca.uky.edu/refcig). CSE KO and C57BL/6 mice (14 weeks old) were placed in whole body exposure chambers and expose to either air or sidestream cigarette smoke (target concentration=1.5mg/m^3). Mice were exposed to the smoke 5 h/day with 30 min smoke free

interval, for 5 days per week for a period of 2 weeks. The smoking apparatus is engineered to collect side stream smoke within a smoking chamber through vacuum suction and drawn into the mixing chamber. Meanwhile mainstream smoke is likewise drawn by vacuum into a water extraction chamber to remove water soluble constituents from the smoke. The extracted smoke is then also passed on to the mixing chamber where it rejoins the side stream smoke where they are mixed together then are passed to the animal exposure chamber. The exposure chamber is of sufficient size to house 60 mice per the period of exposure. These mice were exposed to a total of 10 cigarettes over a 5 hour time frame. Each cigarette was 'smoked' over 5 minute periods at a rate of one 60 ml puff every 30 seconds. After exposure, air was continuously circulated through the exposure chamber until the next exposure time point.

In some experiments, at the end of the two-week exposure period groups of mice were infected intranasally (i.n.) with 50 µl of RSV diluted in phosphate-buffered saline (PBS), at dose 10^6 PFU/mouse or were mock-inoculated using the same volume of PBS (sham infection).

Daily determination of body weight, illness score, cytokine and chemokine concentration and cell differential counts in bronchoalveolar lavage (BAL) fluid, and airway function tests were performed. Mouse strains, side-stream cigarette smoke or air exposure, and infection with RSV or sham are identified in the paper as: WT or CSE KO, TS or Air, RSV or PBS.

Supplemental Figure S1 shows the time line of the experimental protocol (air/smoke exposure as well as RSV or mock infection) with AHR measurements and samples collections.



Supplemental Figure S1. Study protocol. Time line of experimental protocol for **(a)** air/smoke exposure and **(b)** air/smoke exposure followed by either PBS or RSV infection.

Clinical Disease

Animals from all groups were evaluated on a daily basis for weight loss, illness score, and presence of any respiratory symptoms. The percentage of body weight change was plotted over time. A clinical illness score for mice was used to measure the severity of clinical disease (0-healthy; 1-barely ruffled fur; 2-ruffled fur but active; 3-ruffled fur and inactive; 4-ruffled fur, inactive, and hunched; and 5-dead). These parameters have been shown to closely correlate with lung pathology in experimental infection of mice.

Broncholaveolar Lavage

Broncholaveolar lavage was collected *via* the trachea by flushing the lungs twice with 1 ml of ice-cold PBS. A total of 100 µl of BAL fluid was used for cytospin analysis, and the rest was immediately centrifuged and stored at −80°C. Total number of BAL cells was counted with a hemacytometer and viability was assessed by trypan blue. BAL differential cell counts were determined using morphogenic criteria under light microscopy of Protocol HEMA3 (Fisher Scientific) stained cytospins with a total count of 200 cells per slide.

Measurement of Cytokines, Chemokines

Levels of cytokines and chemokines in BAL fluid were determined with the Bio-Plex Pro Mouse Group I 23-plex panel (Bio-Rad Laboratories, Hercules, CA, USA), according to the

manufacturer's instructions. The panel included the following cytokines with the lower limit of quantitation (LLQ): IL-1α (1.84pg/ml), IL-1β (10.36 pg/ml), IL-2 (3.72 pg/ml), IL-3 (1.55 pg/ml), IL-4 (6.98 pg/ml), IL-5 (3.57 pg/ml), IL-6 (0.74 pg/ml), IL-9 (6.89 pg/ml), IL-10 (2.95 pg/ml), IL-12 p40 (1.53 pg/ml), IL-12 p70 (1.62 pg/ml), IL-13 (47.2 pg/ml), IL-17 (2.65 pg/ml), granulocyte-macrophage colony-stimulating factor (GM-CSF) (21.2 pg/ml), gamma interferon (IFN-γ) (1.84 pg/ml), tumor necrosis factor alpha (TNF-α) (5.8 pg/ml), G-CSF (5.1 pg/ml), Eotaxin (257.9 pg/ml) , KC (3.2 pg/ml), MCP-1 (22.4 pg/ml), macrophage inflammatory protein 1α (MIP-1α) (256.2 pg/ml), MIP-1β (3.33 pg/ml) and RANTES (2.78 pg/ml).

RSV Titration of Lung Tissue

Lungs were removed from infected animals at day 5 after RSV infection. Tissue samples were homogenized in 1 ml of Dulbecco's modified Eagle's medium and centrifuged twice at 14,000 rpm for 1 min at 4ºC. Serial twofold dilutions of the supernatant were determined by plaque assay on HEp-2 cells under methylcellulose overlay. Plaques were visualized 5 days later, and virus titers was expressed as log10 PFU/gram tissue.

Pulmonary Histopathology

Mice were euthanized at days 5 post-infection, and the entire lung was perfused, removed, and fixed in 10% buffered formalin following by paraffin embedding. Multiple 4-µm longitudinal cross-sections were stained with hematoxylin and eosin (H&E). The slides were analyzed and scored for cellular inflammation under light microscopy by a board-certified pathologist with expertise in mouse lung, unaware of the infection status of the animals. Two separate grading

systems were used to assess the lung inflammation. The first grading system, measured the percentage of abnormal perivascular spaces in the tissue sections. The second grading system assigned a 0–4 grade based on severity (0 = normal, 4 = severe pathologic changes) to four different parameters: perivasculitis, bronchiolitis, alveolitis, and necrosis. Ten high power fields were examined for each slide, and average grades were compared between groups and analyzed to determine whether observed differences were statistically significant.

Quantitative Real-Time PCR

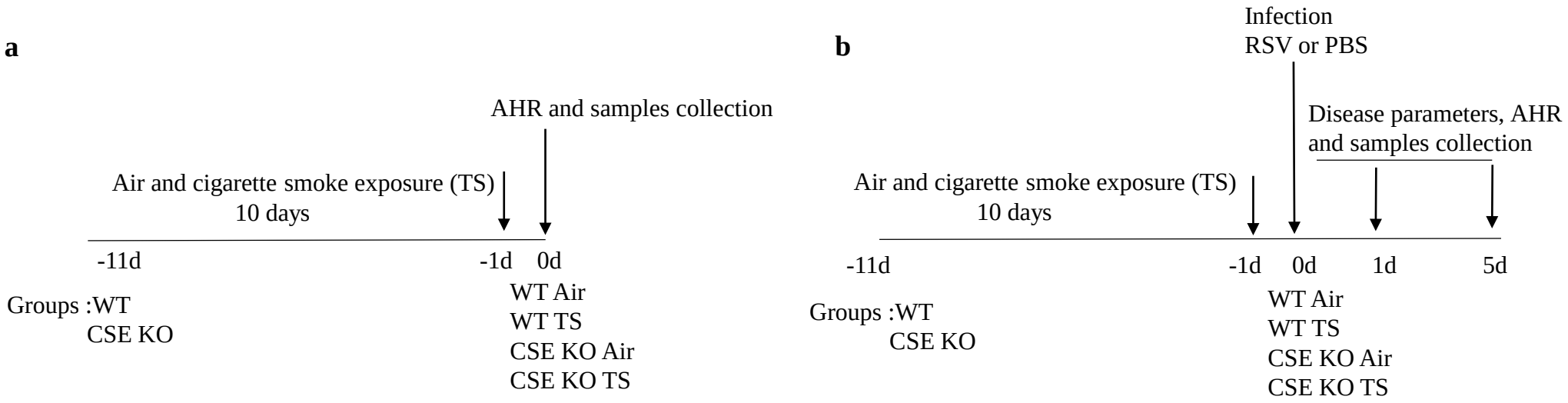
Total RNA was extracted from lung samples by using a ToTALLY RNA kit (catalog number AM1910; Ambion, Austin, TX, USA). RNA samples were quantified by using a NanoDrop spectrophotometer and quality was analyzed on an RNA Nano-drop by using the Agilent 2100 bioanalyzer (Agilent Technologies). Synthesis of cDNA was performed with 1 µg of total RNA in a 20 µl reaction mixture by using the TaqMan Reverse Transcription Reagents kit from ABI (catalog number N8080234; Applied Biosystems). Amplification was done using 1 µl of cDNA in a total volume of 25 µl using the Faststart Universal SYBR green Master Mix (Roche Applied Science #04913850001). The mRNA sequences for CSE, reported under GenBank accession numbers NM_145953 was used to design amplification primers for qRT-PCR assay. 18S RNA was used as housekeeping gene for normalization. PCR assays were run in the ABI Prism 7500 Sequence Detection System. Triplicate cycle threshold (C_T) values were analyzed in Microsoft Excel by the comparative C_T ($\Delta\Delta C_T$) method according to the manufacturer's instructions (Applied Biosystems). The amount of target ($2^{-\Delta\Delta CT}$) was obtained by normalization to the endogenous reference (18S) sample. RNA isolation, primer design, and qRT-PCR assays were performed at Molecular Genomic Core, UTMB, Galveston.

Airway Obstruction and Hyperresponsiveness (AHR)

Air way obstruction and airway hyperresponsiveness was assessed in unrestrained mice at different times after infection using whole-body barometric plethysmography (Buxco™, DSI, New Brighton, MN) to record enhanced pause (Penh). Penh is a dimensionless value that represents a function of the ratio of peak expiratory flow to peak inspiratory flow and a function of the timing of expiration. Respiratory activity was recorded for 5 min, to establish baseline Penh values. Mice were subsequently exposed to increasing doses of nebulized methacholine (3.25, 6.25, 12.5, 25, and 50 mg/ml) for 2 min, and data were recorded for another 3 min.

Supplemental Figure S2. BAL cell differential of RSV-infected mice. TS - or Air-exposed CSE KO and WT mice were infected with RSV or sham. BAL fluid was collected at different time points after infection and cell preparations were stained (Hema 3 stain, Fisher Scientific) and counted under the microscope (200 cells/slide). BAL cell differential expressed as percentage macrophages **(a)**, neutrophils **(b)**, and lymphocyte **(c)**. Data are expressed as mean ± SEM (n = 4 mice (2 male and 2 female)/group and are representative of three independent experiments). *p<0.05.**p<0.01, ***p<0.001. Comparison for statistical analysis was done between groups at day 1post-infection, day 5 post-infection, and day 1 compared with day 5 post-infection. CSE KO TS /RSV vs WT TS / RSV, CSE KO TS / RSV vs CSE KO Air/ RSV; CSE KO Air/RSV vs WT Air/RSV, WT TS / RSV vs WT Air/RSV, CSE KO TS / PBS vs. WT TS / PBS, and CSE KO TS /PBS vs. CSE KO Air/PBS, WT Air/RSV day 1pi vs. WT Air/RSV day 5pi, WT TS/RSV day 1pi vs. WT TS/RSV day 5pi, CSE KO Air/RSV day 1pi vs. CSE KO Air/RSV day 5pi, CSE KO TS/RSV day 1pi vs. CSE KO TS/RSV day 5pi.

Supplemental Figure S1



Supplemental Figure S2