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The Inhibitory Role of Hydrogen Sulfide in Airway Hyperresponsiveness and Inflammation in a Mouse Model of Asthma

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Cystathionine γ-lyase (CSE) is one of the major enzymes producing hydrogen sulfide (H_2S) in lungs, participating in the regulation of respiratory functions. The role of CSE-derived H_2S in eosinophil-dominant inflammation in allergic diseases has been unclear. The objective of this study was to explore the protective role of H_2S against allergen-induced airway hyperresponsiveness (AHR) and inflammation. CSE expression and H_2S production rate were assessed in mouse lung tissues with ovalbumin (OVA)-induced acute asthma. AHR, airway inflammation, and Th2 response in wild-type (WT) mice were compared with those in *CSE* gene knockout (KO) mice. The effect of NaHS, an exogenous H_2S donor, was also evaluated on these parameters. CSE expression was absent and H_2S production rate was significantly lower in the lungs of CSE KO mice when compared with WT littermates. OVA challenge decreased lung CSE expression and H_2S production in WT mice. CSE deficiency resulted in aggravated AHR, increased airway inflammation, and elevated levels of Th2 cytokines such as IL-5, IL-13, and eotaxin-1 in bronchoalveolar lavage fluid after OVA challenge. The aforementioned alterations were reversed by exogenous H_2S treatment. More importantly, NaHS supplement rescued CSE KO mice from the aggravated pathological process of asthma. The CSE/H_2S system plays a critical protective role in the development of asthma. A new therapeutic potential for asthma via targeting CSE/H_2S metabolism is indicated. *(Am J Pathol 2013, 182: 1188–1195; http://dx.doi.org/10.1016/j.ajpath.2012.12.008)*

Hydrogen sulfide (H_2S), traditionally known for centuries as a noxious and toxic gas is now recognized as a third gasotransmitter after nitric oxide and carbon monoxide for its important regulatory roles in chronic respiratory diseases, cardiovascular, and metabolic disorders.[1–5] The role of H_2S in inflammation, however, is still controversial.[2,6] Some studies have showed that exogenous H_2S has a pro-inflammatory effect in various inflammatory models including lipopolysaccharide-induced endotoxemia, acute pancreatitis, and cecal ligation and puncture-induced sepsis.[7–9] Others have reported its anti-inflammatory effects.[10–12] The concentration and the release rate of exogenous H_2S donors are the main factors in determining whether H_2S is anti-inflammatory or pro-inflammatory. The high concentration and fast-releasing rate of H_2S results in a pro-inflammatory effect, and at low concentration and slow-releasing rate H_2S is anti-inflammatory.[12,13]

Asthma is one of the most common chronic inflammatory diseases, and its prevalence has markedly increased throughout the past 2 decades.[14] Eosinophilia and Th2 cytokine production are two hallmarks of allergic asthma.[15] Increased Th2 cytokines, such as IL-4, IL-5, and IL-13, cause immunoglobulin isotype switching of B cells to produce IgE, promote the growth, maturation, and activation of Th2 cells and eosinophils, and result in airway hyperresponsiveness (AHR), mucus production, and airway remodeling.[15] Previous studies have shown the existence of cystathionine γ-lyase (CSE) and/or cystathionine β-synthase (CBS) in peripheral lung tissues of mice and rats.[16,17] Decreased CSE expression and serum H_2S level are observed in the asthma model of rats and in asthmatic

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patients.[17–19] Serum H_2S level is positively correlated with the lung function parameters such as the forced expiratory volume in 1 second, both in adult and pediatric asthma,[18,19] and negatively correlated with sputum count of total cells and the percentage of sputum neutrophils.[18] These results suggest a possible role of endogenous H_2S in the development of asthma. All of these previous studies, however, were based on neutrophil-dominant airway inflammation,[17,18] which is different from eosinophil-dominant airway inflammation in classically allergic asthma.[15] Whether and how endogenous or exogenous H_2S plays a role in the development of eosinophil-dominant inflammation such as allergic asthma and atopic dermatitis remains unknown.

Our previous studies have found that CSE is the main H_2S-producing enzyme in peripheral tissues such as liver, pancreases, and aorta tissues.[3,4] In CSE knockout (KO) mice, endogenous H_2S levels were decreased by 50% in serum, and ~80% in vascular tissues and pancreases.[3,4] Lower level of endogenous H_2S causes hypertension,[4] delays the onset of streptozotocin-induced diabetes,[3] promotes proliferation of smooth muscle cells,[20] and inhibits vascular endothelial growth factor-induced microvessel formation and wound healing[21] in CSE KO mice. To date, the exact role of endogenous H_2S in systemic or local inflammation has not been studied by knocking out the *CSE* gene. Given the critical regulatory role of the CSE/H_2S system in various physiological and pathological conditions, we hypothesized that the endogenous CSE/H_2S pathway may play an important role in the pathogenesis of allergic asthma.

In the present study, we examined the alterations of CSE lung expression and H_2S production, and changes in AHR, airway inflammation, and Th2 response, in CSE KO mice in comparison with wild-type (WT) littermates in a mouse model of asthma. In addition, the effect of exogenous H_2S treatment on the development of asthma was also observed in both WT and CSE KO mice.

Materials and Methods

Mice

CSE KO mice on C57BL/6J × 129SvEv background were generated as we previously described.[4] CSE KO offspring and age-matched male WT littermates were used. All animal experiments were in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and approved by the Animal Care Committee of Lakehead University, Thunder Bay, Ontario, Canada.

OVA-Induced Asthma Model and Experimental Design

WT and CSE KO mice at 8 to 10 weeks were sensitized i.p. with 20 μg chicken ovalbumin (OVA) (Sigma-Aldrich, St. Louis, MO) emulsified in 100 μL Imject Alum (Pierce, Rockford, IL) on days 0 and 7 and then challenged by 100 μg OVA in 40 μL saline administered intranasally after a short anesthesia with isoflurane inhalation on days 28, 29, and 30 (termed as WT-OVA and KO-OVA mice). Sensitized mice were challenged with saline as the control (termed as WT-CON and KO-CON mice). In another group of experiments, mice were given 14 μmol/kg NaHS i.p. (Sigma-Aldrich), 14 μmol/kg NaHS i.p. plus 40 μmol/kg glibenclamide i.p., 20 minutes before NaHS, or vehicle (dimethyl sulfoxide) 30 minutes before and 8 hours after each OVA challenge.

Assessment of AHR

Twenty-four hours after the last OVA challenge, AHR to inhaled methacholine (MCh) (Sigma-Aldrich) was invasively measured. Mice were anesthetized with a combination of 100 mg/kg ketamine hydrochloride and 8.5 mg/kg xylazine, and the tracheas were cannulated and connected to an inline aerosol administrator and ventilator, which were attached to a preamplifier and computer (Buxco, Wilmington, NC). Mice were nebulized with PBS, followed by increasing doses of MCh (3.125, 6.25, 12.5, and 25 mg/mL, respectively). At each dose, the values of lung resistance, reflecting the degree of AHR, were calculated.

Analysis of BALF

A collection of bronchoalveolar lavage fluid (BALF) was performed after AHR measurement. The trachea was cannulated, and inflammatory cells were obtained by lavage of the airway lumen with 0.8 mL saline three times. Cytospin slides were prepared by Wright-Giemsa staining. The number of total and differential cells was counted in a blind fashion.

Lung Histology

Paraffin-embedded lung tissue sections (5 μm) were stained with H&E (Sigma-Aldrich) to evaluate pulmonary inflammatory cell infiltration.

Western Blot

Isolated lung tissues were homogenized and lysed. Equal amounts of proteins were boiled and separated by SDS-PAGE and electrophoretically transferred to nitrocellulose membrane. The dilutions of primary antibodies were 1:1000 for CSE (12217-1-AP; Proteintech Group Inc., Chicago, IL), 1:1000 for CBS (14787-1-AP; Proteintech Group Inc.), and 1:10,000 for β-actin (Sigma-Aldrich). Horseradish peroxidase-conjugated secondary antibodies (Sigma-Aldrich) were used at 1:10,000. The immunoreactions were visualized by enhanced chemiluminescence and exposed to X-ray film (Kodak Scientific Imaging film; VWR International, Mississauga, Canada).

H_2S Production Rate

The measurement of H_2S production rate was previously described.[22] Briefly, isolated lung tissues were homogenized