CCSP modulates airway dysfunction and host responses in an Ova-challenged mouse model

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The present study examines the role of CCSP in the development of airway hyperreactivity (AHR) and airway inflammation in a mouse model of allergic airway disease. CCSP(−/−) and wild-type (WT) mice were preimmunized with ovalbumin (Ova) twice and subsequently challenged with aerosolized Ova by inhalation. CCSP deficiency was associated with increased AHR, increased neutrophil migration into the lungs, and increased levels of Alcian blue (AB)-positive mucosubstances. These findings suggest that CCSP modulates the host response in the lungs of Ova-challenged mice in vivo and provide further evidence for the role of the lung epithelium in regulating airway responses in allergic disease.

MATERIALS AND METHODS

Mice. Female CCSP(−/−) mice (129J Ola/129J hybrid; kindly provided by Dr. Jeffrey A. Whitsett, Children’s Hospital, Cincinnati, OH) and WT (129J; Jackson Laboratory, Bar Harbor, ME) mice were housed under pathogen-free conditions in the Lovelace Respiratory Research Institute.
vivarium following Association for the Assessment and Accreditation of Laboratory Animal Care international guidelines.

**Ova-challenge mouse model.** Ova exposure of mice was modified according to a protocol described previously (32, 35). Briefly, the mice were immunized twice by an intraperitoneal injection of heat-aggregated Ova (10 μg chicken egg, grade V, Sigma, St. Louis, MO) and 2 mg of alum in a total volume of 0.5 ml in endotoxin-free water on days 1 and 8. Preliminary studies indicated that Ova aerosol exposure (5 mg/m³, 6 h/day) for 5 days induced significant airway inflammation in Ova-immunized mice. Therefore, the Ova-immunized mice were then challenged with Ova aerosol (2 or 5 mg/m³, 6 h/day) for 2, 3, or 5 days in whole body exposure chambers (H1000 or H2000; Hazleton Systems, Aberdeen, MD) beginning on day 15. Food and water were available ad libitum.

**Measurement of airway reactivity.** Airway reactivity (AR) was measured with a whole body plethysmograph (Buxco Electronics, Sharon, CT). As previously described (23), AR has been expressed as enhanced pause, a calculated value showing a strong correlation with airway resistance measured with standard procedures (4). Individual mice were placed in parallel chambers connected to a Hudson nebulizer (Micro Mist, Hudson Respiratory Care, Temecula, CA) and a recording system. The nebulizer was driven by stable airflow at a level of 4.42 l/min. The baseline AR of the mice was recorded for 5 min. Subsequently, the mice were challenged for 1 min to nebulized saline and increasing concentrations (6, 12, 25, and 50 mg/ml) of nebulized methacholine (MCh; ICN Biomedicals, Aurora, OH). After each nebulization, recordings were taken for 10 min. The enhanced pause values measured during the first 5 min were averaged and expressed as means ± SE (n = 10 mice/group).

**Cell counts and cytology in BALF.** BALF (n = 10 mice/group) was obtained by three serial intratracheal instillations of 1 ml of PBS into the lung, and the samples were pooled for each animal. Cells in the BALF were isolated by centrifugation at 1,500 rpm and resuspended in 200 µl of PBS. Viable cells were counted by hemacytometer in a 1:1 ratio to the entry of the mainstream bronchus, for paraffin embedding. Paraffin-embedded lungs were sectioned at 5 µm and stained with hematoxylin and eosin for histological analysis. For each animal, slides of the left lung, with two sections of the apical, intermediate, and intermediate lobes of the right lungs (6–8 mice/group) were stained with hematoxylin and eosin for histological analysis. For each animal, slides of the left lung, with two sections of the apical, intermediate, and intermediate lobes of the right lungs (6–8 mice/group) were stained with hematoxylin and eosin for histological analysis.

**Pulmonary histopathology.** Lung inflammation was assessed histopathologically as described (6). Blood was collected by right ventricular puncture. Left lungs were inflated via a tracheal cannula at 20 cmH₂O of pressure with 4% paraformaldehyde and removed en bloc from the thorax. Inflation-fixed lungs were washed in PBS three times and bisected transversely in the dorsoventral direction, just caudal to the entry of the mainstream bronchus, for paraffin embedding. Paraffin-embedded lungs were sectioned at 5 µm and stained with hematoxylin and eosin for histological analysis. For each animal, slides of the left lung, with two sections of the apical, intermediate, and intermediate lobes of the right lungs (6–8 mice/group) were stained with hematoxylin and eosin for histological analysis.

**Mucous cell staining.** Mucous cell stained with AB-periodic acid-Schiff (PAS) stain to identify mucus-secreting cells as described (31). Briefly, the lungs were deparaffinized in xylene and hydrated in decreasing concentrations of ethanol. The slides were then stained in AB for 30 min, washed in running water for 5 min, oxidized in 1% periodic acid for 10 min, and washed in running water for another 5 min. After staining in Schiff’s reagent for 10 min, slides were rinsed three times in sodium metabisulfite, washed in running water for 10 min, and after dehydration, were mounted in ethanol and xylene. Photomicrographs (×415) showed the mucus-producing cells with distinctive colors for PAS positivity (pink) and AB-PAS positivity (purple). In all mice, only the mucous cells in the epithelia lining the main airway were analyzed. The length of the basol lamina lining the large airways of WT and CCSP(−/−) mice and the volume density of the AB-PAS-stained mucosubstances in the surface epithelium were determined with an Everest microscope system equipped with slidebook software (Intelligent Imaging Innovations, Denver, CO) as described previously (5).

**Statistical analysis.** Statistical significance between groups was determined by t-test for parametric data or Mann-Whitney test for nonparametric data. Statistical analysis for multiple groups was determined by ANOVA. All data are presented as means ± SE. Differences were considered significant at P < 0.05.

**RESULTS**

**AR is increased in CCSP(−/−) mice after Ova exposure.** To assess the role of CCSP in modulating the development of AHR induced by Ova, CCSP(−/−) and WT mice (8–9 wk of age at the time of AR measurement) were immunized twice by a weekly intraperitoneal injection of Ova-alum. On day 15, the mice were challenged with Ova aerosol (2 or 5 mg/m³) for 2, 3, or 5 days (6 h/day). The WT air control mice responded very poorly to all doses of MCh (Fig. 1). The AR in CCSP(−/−) air control mice was not different from that in WT air control mice at all doses of MCh challenge.
mice challenged with 2 mg/m$^3$ of Ova compared with that in wild-type (WT) mice after exposure to 2 mg/m$^3$ of ovalbumin (Ova) for 2, 3, and 5 days (d). Baseline AR was recorded in 4 groups of mice: WT air control (WT Air), WT Ova challenge (WT Ova), CCSP(-/-) air control [CCSP(-/-) Air], and CCSP(-/-) Ova challenge [CCSP(-/-) Ova]; n = 10/group. AR (enhanced pause; $P_{enh}$) is expressed as $P_{enh}$ values (means ± SE). Open symbols, AR in CCSP(-/-) mice; closed symbols, AR in WT mice. *Significant difference between WT 2d (Ova) and WT Air groups at 25 mg/ml of methacholine (MCh) challenge. **Significant difference between WT 2d and WT 3d (Ova) and WT Air groups at 50 mg/ml of MCh challenge. ***Significant difference in AR in CCSP(-/-) Ova 2d, 3d, and 5d compared with WT Ova 2d, 3d, and 5d, respectively, at both 25 and 50 mg/ml of MCh challenge.

The AR in WT mice challenged with 25 mg/ml of MCh was only increased after 3 days of Ova exposure at 2 mg/m$^3$ and increased at 50 mg/ml of MCh challenge after 2 or 3 days of Ova exposure at 2 mg/m$^3$ compared with that in WT air control mice (Fig. 1). In contrast, the AR of CCSP(-/-) mice challenged at both 25 and 50 mg/ml of MCh was increased after 2, 3, and 5 days of Ova exposure at 2 mg/m$^3$ compared with that of WT mice exposed to Ova at 2 mg/m$^3$ for 2, 3, and 5 days, respectively. The AR of both WT and CCSP(-/-) mice exposed to 5 mg/m$^3$ of Ova (data not shown) was similar to that of mice exposed to 2 mg/m$^3$ of Ova. Overall, the AR of CCSP(-/-) mice after Ova exposure was increased compared with that in WT mice after 2, 3, and 5 days of Ova exposure at either 2 (Fig. 1) or 5 mg/m$^3$ of Ova aerosol.

Inflammation is increased in the lungs of CCSP(-/-) mice challenged with Ova. To determine the role of CCSP in modulating inflammatory responses in lung tissues, pathological assessment of lung inflammation and injury was scored blindly according to septal infiltrates, perivascular infiltrates, peribronchial infiltrates, and epithelial cell hyperplasia/hypertrophy in CCSP(-/-) mice and WT mice after Ova challenge. The mean pathological scores were markedly increased in both WT and CCSP(-/-) mice challenged with 2 mg/m$^3$ of Ova compared with scores in air control mice. The mean pathological scores of CCSP(-/-) mice were increased compared with those of WT mice after Ova challenge at 2 mg/m$^3$ for 2 and 3 days (Fig. 2A). No statistical difference was observed between CCSP(-/-) and WT mice after Ova exposure at 5 mg/m$^3$ for 2 and 3 days, but lung histopathology was increased in CCSP(-/-) mice exposed to Ova for 5 days. Representative photomicrographs show the histopathological changes in the lungs of WT and CCSP(-/-) mice challenged with 2 mg/m$^3$ of Ova for 3 days (Fig. 2B).

Cell differential analysis in BALF of CCSP(-/-) mice after Ova challenge. Differential cell analysis was assessed in BALF of CCSP(-/-) mice after Ova exposure to determine the role of CCSP in modulating inflammatory cell populations in the lung. Eosinophils were the predominant inflammatory cells in the BALF of all mice challenged with Ova even after 2 days of exposure. Eosinophils in BALF from CCSP(-/-) mice were increased compared with WT mice after Ova challenge at 5 mg/m$^3$ for 5 days (Fig. 3) but were not altered at 2 or 3 days or in 2 mg/m$^3$ Ova-exposed CCSP(-/-) mice at 2, 3, or 5 days. The numbers of neutrophils in BALF from WT mice were increased 2 and 3 days after Ova challenge compared with numbers in WT air control mice. Neutrophils in BALF from CCSP(-/-) mice were further increased compared with those in WT mice after 2 and 3 days of Ova challenge at both 2 and 5 mg/m$^3$ (Fig. 4). In contrast to eosinophils, which increased later in CCSP(-/-) mice after Ova exposure compared with WT mice, neutrophils increased early during the course of Ova exposure in CCSP(-/-) mice and were decreased after 5 days of exposure. Lymphocytes in BALF from both WT and CCSP(-/-) mice after 2 or 5 mg/m$^3$ of Ova exposure were increased compared with levels in air control mice. The numbers of lymphocytes in BALF from CCSP(-/-) mice were increased compared with those in WT mice after 2 or 5 mg/m$^3$ of Ova exposure for 5 days (Fig. 5). Macrophages were increased in BALF of both WT and CCSP(-/-) mice exposed to Ova at 2 mg/m$^3$ for 2 and 3 days and 5 mg/m$^3$ for 2, 3, and 5 days compared with macrophages in air control mice. However, there was no statistical difference between macrophage counts in BALF of WT and CCSP(-/-) mice at all dose and time points (data not shown). Overall, neutrophils were increased in the BALF of CCSP(-/-) mice after 2 and 3 days of Ova exposure at either 2 or 5 mg/m$^3$, whereas eosinophils were increased in the BALF from CCSP(-/-) mice only after 5 days of Ova challenge at 5 mg/m$^3$. Lymphocytes were increased in the BALF from CCSP(-/-) mice after 5 days of Ova exposure at both 2 and 5 mg/m$^3$.

MPO activity is increased in the lungs of CCSP(-/-) mice after Ova exposure. As a marker of neutrophil activation during Ova-induced lung inflammation, MPO activity was measured in lung homogenates and BALF of CCSP(-/-) and WT mice after Ova challenge. MPO activity was undetectable in the original BALF and the concentrated BALF from WT and CCSP(-/-) mice. MPO activity was also undetectable in both WT and CCSP(-/-) air control mice. MPO activity was increased in WT mice after Ova challenge compared with that in WT air control mice (Fig. 6). MPO activity was highest in the lungs of mice after 2 days of Ova exposure and declined with time. MPO activity was only mildly apparent after 5 days of Ova exposure at 2
or 5 mg/m\(^3\). MPO activity was further increased in the lungs of CCSP\((-/-)\) mice compared with that in WT mice after 2 or 3 days of Ova challenge at 2 mg/m\(^3\) of Ova aerosol, consistent with the increased numbers of neutrophils in the BALF from CCSP\((-/-)\) mice. After 5 mg/m\(^3\) of Ova challenge, MPO activity was increased in the lung homogenates of CCSP\((-/-)\) mice compared with that in WT mice after 2 days of Ova exposure. Interestingly, MPO activity was similar in the lung homogenates from CCSP\((-/-)\) and WT mice after 3 days.

**Fig. 2.** Histopathological scores were increased in the lungs of CCSP\((-/-)\) mice compared with those in WT mice after Ova challenge. Pathological assessment of lung inflammation and injury was scored in mice exposed to 2 mg/m\(^3\) of Ova for 2, 3, and 5 days (n = 6–8/group). Air control mice were also assessed and scored as 0. Data are means ± SE. *A: histopathological scores were increased in CCSP\((-/-)\) mice compared with those in WT mice after 2 or 3 days of Ova exposure (\(P < 0.05\)). B: photomicrographs were taken from representative slides of WT and CCSP\((-/-)\) mice challenged with 2 mg/m\(^3\) of Ova for 3 days.

**Fig. 3.** Eosinophils were increased in bronchoalveolar lavage fluid (BALF) from CCSP\((-/-)\) compared with WT mice after 5 days of exposure to 5 mg/m\(^3\) of Ova. Total eosinophils in BALF were counted after either air or Ova exposure (2 or 5 mg/m\(^3\)) for 2, 3, or 5 days (n = 10/group). Data are means ± SE of total eosinophil counts in BALF. Eosinophils were only increased in CCSP\((-/-)\) mice compared with those in WT mice after Ova challenge at 5 mg/m\(^3\) for 5 days (\(P < 0.05\)).

**Fig. 4.** Number of neutrophils in BALF was significantly increased in CCSP\((-/-)\) mice compared with that in WT mice after Ova exposure for 2 or 3 days. Total neutrophils in BALF were counted after either air or Ova challenge (n = 10/group). Mice were exposed to Ova at 2 or 5 mg/m\(^3\) for 2, 3, or 5 days, respectively. Data are means ± SE of total neutrophil counts in BALF. Neutrophil counts in CCSP\((-/-)\) Ova mice were significantly increased compared with those in WT Ova mice exposed to 2 mg/m\(^3\) of Ova for 2 and 3 days (\(P < 0.05\)) and 5 mg/m\(^3\) of Ova for 2 (\(\approx P < 0.01\)) and 3 (\(P < 0.05\)) days.
days of exposure at 5 mg/m³ of Ova aerosol. Collectively, MPO activity was increased in the lungs of CCSP(−/−) mice early during the course of Ova-induced lung inflammation and coincided with increased neutrophils in the lungs of CCSP(−/−) mice after Ova challenge.

Increased MIP-2 concentration in lungs from CCSP(−/−) mice exposed to Ova. To determine a possible mechanism by which CCSP deficiency causes increased neutrophilic recruitment in BALF and increased lung infiltration, the neutrophil chemokine MIP-2 was assessed in lung homogenates of CCSP(−/−) and WT mice. MIP-2 levels were not readily detectable in either WT or CCSP(−/−) air control mice. MIP-2 levels were increased in the lungs of WT mice after 2, 3, or 5 days of Ova challenge compared with levels in WT air control mice (Fig. 7, A and B). In the lungs of CCSP(−/−) mice, MIP-2 levels were increased twofold compared with those in WT mice after 2 and 3 days of Ova challenge with 2 mg/m³ of Ova aerosol (Fig. 7A). Furthermore, the MIP-2 levels were increased in the lung homogenates of CCSP(−/−) mice at all time points after Ova exposure at 5 mg/m³ (Fig. 7B). Overall, MIP-2 production was increased in the lungs of CCSP(−/−) mice after Ova challenge, in accordance with increased neutrophil migration in the lung.

Increased AB-PAS-positive mucosubstances in CCSP(−/−) mice challenged with Ova. The role of CCSP in modulating Ova-induced mucous cell metaplasia was examined by AB-PAS staining of the airways in CCSP(−/−) and WT mice. Mucus staining was not apparent in the airway epithelium of WT or CCSP(−/−) air-challenged mice (Fig. 8A). Very few AB-PAS-positive cells were visible in CCSP(−/−) and WT mice after 2 or 3 days of challenge with both 2 and 5 mg/m³ of Ova aerosol (data not shown). The intensity and the number of AB-PAS-stained cells increased after 5 days of challenge with 2 and 5 mg/m³ of Ova aerosol compared with results at 2 or 3 days of chal-
challenge. Many of the mucous cells in the WT mice showed only PAS positivity, whereas many mucous cells in CCSP(−/−) mice displayed AB-PAS-positive staining (Fig. 8A). Although the numbers of AB-PAS-positive cells per millimeter of basal lamina were similar in WT and CCSP(−/−) mice (data not shown), the volume density of AB-positive material was significantly higher in CCSP(−/−) mice compared with WT mice after Ova challenge at 5 mg/m³ for 5 days (Fig. 8B).

**DISCUSSION**

The role of CCSP and Clara cells in the pathogenesis of allergic airway disease has not been elucidated. In the present study, AR was increased in CCSP(−/−) mice after Ova aerosol challenge. Lung inflammation was increased in the lungs of CCSP(−/−) mice concurrent with increased AR. Neutrophils appeared earlier and were increased during the pathogenesis of Ova-induced lung disease in CCSP(−/−) mice. Likewise, eosinophils and lymphocytes were also increased at later time points. MPO activity and MIP-2 levels were increased in CCSP(−/−) mice after Ova challenge, coinciding with increased neutrophils in the lungs of CCSP(−/−) mice. Furthermore, the amount of AB-PAS-positive mucosubstances was increased in the airways of CCSP(−/−) mice after Ova exposure. These results indicate that CCSP modulates AHR and lung inflammation induced by Ova exposure and are consistent with the concept that CCSP limits the pathogenesis of asthma and allergic airway disease.

Herein, AR was markedly higher in CCSP(−/−) mice than in WT mice after Ova challenge. The role of CCSP in regulating airway responses during airway disease has not been investigated. Previous studies indicate that CCSP levels are decreased in BALF of patients with chronic bronchitis (9) and in animal models of acute bacterial infection (7). In a recent clinical study (24), CCSP-positive epithelial cells were reduced in the...
small airways of patients with asthma, and CCSPpositive epithelial cell proportions were inversely correlated with numbers of T cells and mast cells in the small airways of these patients. Likewise, CCSP levels in BALF (33) and serum (25) are also decreased in asthmatic patients. Taken together, these studies suggest that CCSP is decreased during chronic airway diseases, particularly asthma. The present study supports the concept that CCSP deficiency in the lung can contribute to the development of AHR and the pathogenesis of asthma.

In the present study, either 2 or 5 mg/m$^3$ of Ova exposure for 6 h/day caused increased AR and lung inflammation characteristic of asthma. Lung inflammation and AR were increased in the lungs of CCSP$^{-/-}$ mice compared with WT mice. A previous study (2) with 129J strain mice and acute Ova exposure (either 20 or 60 min/day) reported poor host responses to Ova challenge. In the present study, a 6-h exposure of WT 129J strain mice to Ova aerosol induced mild AR at high concentrations (25 or 50 mg/ml) of MCh challenge. Importantly, the AR was further increased in CCSP$^{-/-}$ mice exposed to Ova for 2, 3, or 5 days at either 25 or 50 mg/ml of MCh challenge compared with that in WT mice, indicating that CCSP deficiency can render 129J strain mice susceptible to Ova challenge. The measurement of Ova concentrations in the exposure chambers strengthens the experimental protocol used herein and limits variations between experimental studies.

Neutrophils were increased early during the course of Ova-induced lung disease in the lungs of CCSP$^{-/-}$ mice compared with numbers in WT mice. The role of CCSP in modulating neutrophil migration into the lung has been shown. Neutrophils in BALF from CCSP$^{-/-}$ mice are markedly increased after acute adenovirus or Pseudomonas aeruginosa infection (6, 7). Likewise, lung neutrophils are increased in CCSP$^{-/-}$ mice during the course of Ova-induced lung disease in vivo. Neutrophilia in the airways has been associated with severe asthma (22), and neutrophils can damage airway epithelial cells in airway inflammation (34). Thus increased neutrophils early during Ova-induced airway disease in CCSP$^{-/-}$ mice may, in part, account for the increased AR observed in CCSP$^{-/-}$ mice after Ova exposure. In the present study, MPO activity, an important effector of neutrophil-induced cytotoxicity, was also increased in the lungs of CCSP$^{-/-}$ mice during the allergic response to Ova. Importantly, MPO activity was measured in total lung homogenates as an index of neutrophils in the lungs after Ova exposure. Thus extracellular MPO activity cannot be distinguished from intracellular stores of MPO. The contribution of intravascular MPO within lung blood vessels is likely small due to exsanguination of tissues during preparation. Therefore, the increased MPO activity in the lung homogenates of CCSP$^{-/-}$ mice after Ova challenge suggests that MPO-containing leukocytes accumulated more in the lungs of CCSP$^{-/-}$ than in WT mice after Ova challenge. Collectively, these results strongly suggest a role for CCSP in modulating neutrophil or MPO-containing leukocyte responses in the lung after allergic challenge.

In contrast to neutrophils, which increased earlier during the course of Ova challenge, lymphocytes increased in the BALF of CCSP$^{-/-}$ mice after 5 days of Ova exposure at both 2 and 5 mg/m$^3$. However, eosinophils increased in the BALF of CCSP$^{-/-}$ mice after 5 days of Ova exposure only at 5 mg/m$^3$, not at 2 mg/m$^3$. The findings in the current study suggest an Ova dosage-dependent infiltration of eosinophils into the airways of sensitized mice, indicating that a larger dose of allergen challenge could induce more severe leukocyte, especially eosinophil, infiltrates in the lung. This finding is similar to the observations from a study in human patients with atopic asthma in which only subjects challenged with a high dose of antigen recruited significant eosinophils to the lung (3).

Lung histopathology was increased earlier in CCSP$^{-/-}$ mice during the course of Ova-induced lung pathogenesis. Increased lung infiltration coincided with increased neutrophils, increased MIP-2 production, and MPO activity in the lungs of CCSP$^{-/-}$ mice after Ova challenge. Likewise, the increased lung inflammation coincided with increased AR in CCSP$^{-/-}$ mice. In previous studies, lung inflammation was increased in CCSP$^{-/-}$ mice after acute respiratory infections (6, 7, 10). Lung inflammation and injury also increased in CCSP$^{-/-}$ mice exposed to ozone and hyperoxia (12, 15). Inflammation of the lung is closely associated with AHR in both experimental animal models and clinical studies. Undoubtedly, inflammatory cells in the lung secrete a number of mediators that are involved in AR to allergens and are strongly associated with the development of asthmatic responses. Taken together, these studies suggest that CCSP modulation of lung inflammation may reduce AR in Ova-induced lung disease in vivo.

The migration of specific inflammatory cells into the lung is a complex mechanism involving, in part, chemotaxis by various effector molecules. MIP-2 levels were increased in the lungs of CCSP$^{-/-}$ mice compared with those in WT mice after Ova challenge. In the present study, MIP-2 levels were measured in total lung homogenates. As with the measurements of MPO, intravascular MIP-2 could not be distinguished from MIP-2 in lung interstitium in the present study. In mice, MIP-2 is an important chemoattractant for neutrophils, both in vitro and in vivo (1, 17). In the present study, increased MIP-2 levels in the lung coincided with increased neutrophil migration in the airways and lung parenchyma. In a previous study (6) of CCSP deficiency, MIP-2 was increased in the lungs of CCSP$^{-/-}$ mice after acute viral infection. Increased lung MIP-2 levels in virally infected CCSP$^{-/-}$ mice strongly coincided with increased neutrophils in the lung as is the case herein. Likewise, various chemokines, in particular MIP-2, are increased in the lungs of CCSP$^{-/-}$ mice during oxygen- or ozone-induced lung injury (11). These findings suggest that CCSP may play an important role in modulating chemokine production in the lung and may provide the basis for the
presence of increased neutrophils in the lungs of CCSP-deficient mice.

The role of CCSP in mucus production has not been previously investigated. After Ova exposure, both CCSP(-/-) and WT mice exhibited similar numbers of AB-PAS-positive cells. However, more AB-positive material was detected in the airways of CCSP(-/-) than in WT mice. The change in staining pattern denotes a change in the content from neutral (PAS-positive) to acidic (AB-positive) glycoprotein (21). Clara cells have been shown to differentiate into mucus-containing cells in the airways of mice (16, 36). It is possible that after allergen exposure, the changes in inflammatory responses in CCSP(-/-) mice led to the expression of different glycosylating mechanisms and thereby to a different pattern of glycosylation.

In the current study, CCSP deficiency was associated with increased lung pathology and inflammation after Ova challenge. A previous study (29) in CCSP(-/-) mice showed that Clara cell ultrastructures are also altered in this animal model. The significance of this observation after Ova aerosol challenge herein is unclear. Importantly, CCSP replacement with recombinant CCSP in the lungs of CCSP(-/-) mice attenuates lung inflammation and cytokine production in the lung after lipopolysaccharide challenge (8), suggesting that the absence of CCSP was, in part, involved in the increased host response to lipopolysaccharide in CCSP(-/-) mice. Surfactant mRNA production is not altered in CCSP(-/-) mice (29), suggesting that Clara cells retain normal surfactant production in CCSP(-/-) mice. Likewise, surfactant homeostasis is not altered in the lungs of uninfected or adenovirus-infected CCSP(-/-) mice (10). The current study does not exclude other functions of the Clara cells secondary to CCSP deficiency that contribute to the present findings. Importantly, the dosimetry of aerosol deposition is not altered in the lungs of CCSP(-/-) mice (Stripp BR, personal communication). The current studies strongly suggest a role for CCSP, whether direct or indirect, in modulating lung inflammation and AHR in response to allergic airway inflammation.

CCSP in vivo modulates lung inflammation during environmental injury and acute infection. The present study supports the concept that CCSP modulates lung inflammation and airway responsiveness to inhaled allergens in vivo and suggests a role for Clara cells in the pathogenesis of asthma. CCSP is decreased in the airways of patients with asthma (23, 32). Thus decreased CCSP may have important implications in the development of chronic lung inflammation in asthmatic and atopic diseases.

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