Clara cell secretory protein decreases lung inflammation after acute virus infection

KEVIN S. HARROD,1 AMBER D. MOUNDAY,1 BARRY R. STRIPP,2 AND JEFFREY A. WHITSETT1

1Division of Pulmonary Biology, Children's Hospital Medical Center, Cincinnati, Ohio 45229; and 2Department of Environmental Medicine, University of Rochester, Rochester, New York 14642

Harrod, Kevin S., Amber D. Mounday, Barry R. Stripp, and Jeffrey A. Whitsett. Clara cell secretory protein decreases lung inflammation after acute virus infection. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19): L924–L930, 1998—Clara cell secretory protein (CCSP) is an abundant 10-kDa polypeptide synthesized and secreted primarily by nonciliated bronchiolar epithelial cells in the mammalian lung. To determine the potential role of CCSP in pulmonary inflammation after acute viral infection, CCSP gene-targeted (CCSP-deficient [CCSP(−/−)]) mice were exposed to a recombinant adenoviral vector, Ad5Luc1, intratracheally. Lung inflammation was markedly increased in CCSP(−/−) mice compared with wild-type control mice and was associated with an increased number of polymorphonuclear cell infiltrates and epithelial cell injury in both conducting airways and alveolar regions. Histological evidence of pulmonary inflammation in CCSP(−/−) mice was associated with increased production of cytokine (interleukin-1α and -6 and tumor necrosis factor-α) mRNA and protein, as well as chemokine (macrophage inflammatory protein-1α and -2 and monocyte chemotactic protein-1) mRNA expression within the lung in response to adenoviral infection. Adenoviral-mediated gene transfer was decreased in CCSP(−/−) mice relative to wild-type mice as measured by luciferase enzyme activity in lung homogenates. The present study suggests that CCSP is involved in modulating lung inflammation during viral infection and supports a role for CCSP in lung host defense.

Lung inflammation after viral infection with adenoviruses and adenoviral vectors has been well characterized. Adenoviruses are ubiquitous viral pathogens that cause respiratory, gastrointestinal, and genitourinary infections (16). Adenoviruses usually cause acute respiratory pathology; however, adenoviruses can also persist as asymptomatic infections of the respiratory tract (16). Acutely, adenoviral infection causes lung infiltration of macrophages and neutrophils in the alveolar air spaces, generally observed 2–3 days after infection (2). Concentrations of the cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-2 are also increased in pulmonary tissues after adenoviral infection, coinciding with the appearance of macrophages in alveolar regions (2). Because of their tropism for airway epithelial cells, adenoviruses have been utilized as vectors for gene transfer to the lung. The efficiency and duration of gene expression with recombinant adenoviruses, however, are limited by host inflammatory and immune responses (1, 18, 19, 22). Thus lung inflammation after infection with adenoviral vectors has been well characterized and provides a useful model for studying viral-induced inflammation in the lung.

Although the role of CCSP in infectious injury in the lung has not been addressed, this report tests whether CCSP modulates host responses to viral infection in the lung. CCSP(−/−) mice were infected with a replication-deficient adenoviral vector, and lung inflammation and acute immune responses were measured. The deficiency of CCSP in mice increases lung inflammation after intratracheal administration of an adenovirus.

MATERIALS AND METHODS

Mice CCSP(−/−) [129IOLA/129I hybrid] and wild-type 129I (Taconic Farms, Germantown, NY) mice were housed under pathogen-free conditions in the Children's Hospital (Cincinnati, OH) Research Foundation vivarium as required by American Association for Accreditation of Laboratory Animal Care guidelines.

Intratracheal administration of adenovirus. Eight- to twelve-week-old CCSP(−/−) and 129I wild-type control mice (n = 6–12 mice/group) were used. The procedure for intratra-
VIRAL INFECTION IN CCSP-DEFICIENT MICE

L925

chel administration of adenoviral vectors was previously described by Zsengeller et al. (22). Briefly, mice were anesthetized with methoxyflurane vapor, and a ventral midline incision was made to expose the trachea. Intratracheal inoculation of $1 \times 10^8$ plaque-forming units of Av1Luc1, an E1- and E3-deleted adenoviral vector expressing firefly luciferase from the Rous sarcoma virus promoter, in 100 µl of delivery vehicle (10 mM Tris, 1 mM MgCl₂, and 10% glycerol, pH 7.4) was performed with a bent, 27-gauge tuberculin syringe (Monoject, St. Louis, MO). The incision was closed with one drop of Xenaband liquid, and the mice were allowed to recover. Mice recover rapidly and remain active after the procedure. At a predetermined time of biological analysis, the mice were killed by a lethal injection of pentobarbital sodium. A midline incision was made in the abdomen. Exsanguination was accomplished by transection of the inferior vena cava to reduce hemorrhage in the lung. For histological studies, RNA and protein analyses, the right upper, middle, and lower lobes of the lung were damped with a hemostat and removed for measurement of luciferase activity, RNA, and protein. The left lung lobe was inflated with 4% paraformaldehyde (Electron Microscopy Sciences, Ft. Washington, PA) and fixed overnight for histological examination.

Evaluation of acute inflammatory cell infiltrates. Inflammatory cell numbers and percentages were evaluated at 4 and 24 h after adenoviral vector administration. Bronchoalveolar lavage (BAL; n = 6 mice/group) fluid was obtained by intratracheal instillation of 1 ml of PBS into the lung while it was maintained within the thoracic cavity. The lavage was rein fused into the lung two times before final collection. BAL cells were isolated by centrifugation at 500 g and resuspended in 500 µl of PBS, 100 µl of the cell suspension were mixed in 100 µl of 0.4% trypan blue (GIBCO BRL, Grand Island, NY), and the cells were counted with a hemocytometer. To determine inflammatory cell types in BAL, 5 x 10³ cells were counted on slides by cytospin centrifugation in 100 µl of PBS at 600 rpm for 3 min. Cell types were identified and counted by differential staining microscopy with Diff-Quik (Baxter Healthcare, Miami, FL). Inflammatory cell populations were determined by counting 100 cells, and a percentage was calculated based on five sample sets from three animals per group.

Cytokine analysis. Cytokine mRNA abundance was determined by RT-PCR analysis of whole lung total RNA. Briefly, whole lung total RNA was isolated by phenol-chloroform extraction and precipitation with isopropanol and treated with the Phased-Lock protocol (5 Prime — 3 Prime, Boulder, CO). Total RNA quantitation was confirmed by gel electrophoresis. Total RNA was converted to cDNA by the RT reaction (GIBCO BRL, Gaithersburg, MD). PCR for cytokine cDNA was performed with the following primer tandems: IL-1β primer 1, 5'-TGTCCTGCTTCATGAC-ACCTA-3' and primer 2, 5'-CTTCTTGGATCAGCGACGTGTT-TC-3'; IL-6 primer 1, 5'-GGCCTCGTCTGCTGATGCT-3' and primer 2, 5'-GTATCCTCTGAAAGGACCTGTT-3'; TNFα primer 1, 5'-CCAGACCTCACACCTGAT-3' and primer 2, 5'-AACACCATCCTTACCTGAG-3'; IFNγ primer 1, 5'-AGCCAGCCGTGTTGACACGGT-3' and primer 2, 5'-GGTGGCTCGGAGGAGGGAAG-3'; IL-12 primer 1, 5'-AGACAGACCTACACTGAT-3' and primer 2, 5'-AACACCATCCTTACCTGAG-3'; IL-10 primer 1, 5'-GGTGCGCTGGGGTCTGAGC-3' and primer 2, 5'-GGGCGCTGGGGTCTGAGC-3'; IL-4 primer 1, 5'-GACAGACCTACACTGAT-3' and primer 2, 5'-AACACCATCCTTACCTGAG-3'; IL-2 primer 1, 5'-TCCTTGTGAGGAGGAGGGAAG-3' and primer 2, 5'-GGCCTCGTCTGCTGATGCT-3'.

Statistical analysis. Statistical analysis for multiple groups was determined by ANOVA with StatWorks computer software. All data are presented as means ± SE. Differences were considered significant at P < 0.05.

RESULTS

BAL cell counts are increased in CCSP(-/-) mice. To assess the role of CCSP in modulating lung inflammatory cell infiltrates after adenoviral infection, adult CCSP(-/-) mice (in 129J strain background) and 129J wild-type mice (7–15 wk of age) were intratracheally injected with a recombinant, replication-deficient (E1- and E3-deleted) adenoviral vector, Av1Luc1. Inflammatory cells in BAL fluid were assessed at 4 and 24 h postinfection for cell counts in BAL fluid. Both wild-type (129J) and CCSP(-/-) mice tolerated 10⁹ plaque-forming units of adenovirus and were responsive and active within 1 h of the surgical procedure. No mortality was observed after administration of the virus to either group of mice. At 4 h after adenoviral infection, cell counts in BAL fluid from control 129J mice were not different from cell counts in uninfected mice (Fig. 1A). Four hours after Av1Luc1 administration, cell counts in BAL fluid from CCSP(-/-) mice were increased three-fold compared with those in wild-type mice. Twenty-four hours after adenoviral infection, cell counts in BAL fluid from wild-type mice were increased compared with cell counts in BAL fluid from uninfected wild-type mice. Cell counts in BAL fluid from infected CCSP(-/-) mice at 24 h were increased 3.5-fold compared with those in infected wild-type mice. Cell counts in BAL fluid from infected mice did not vary in either group. The number of cells obtained from BAL in CCSP(-/-) and wild-type mice were similar before infection.

To determine whether individual inflammatory cell populations were altered in BAL fluid from CCSP(-/-) mice, BAL fluid was obtained from adenoviral-infected...
wild-type and CCSP(-/-) mice and examined after cytospin centrifugation and differential cell staining. Four hours after adenoviral infection, inflammatory cells in BAL fluid from wild-type mice were predominantly macrophages and were not different from those in BAL fluid from uninfected mice (Fig. 1B, Table 1).

Table 1. Differential cell counts in BAL from wild-type and CCSP(-/-) mice after adenoviral infection

<table>
<thead>
<tr>
<th></th>
<th>Mac</th>
<th>PMN</th>
<th>Lym</th>
<th>Mac</th>
<th>PMN</th>
<th>Lym</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
<td>24 h</td>
<td></td>
<td>4 h</td>
<td>24 h</td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>92.6 ± 1.4</td>
<td>5.2 ± 1.0</td>
<td>2.1 ± 0.7</td>
<td>87.5 ± 2.1</td>
<td>11.4 ± 1.8</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>CCSP(-/-)</td>
<td>76.8 ± 1.6*</td>
<td>19.8 ± 1.8*</td>
<td>3.3 ± 1.3</td>
<td>64.3 ± 1.6*</td>
<td>33.2 ± 1.8*</td>
<td>2.5 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE in percent; n = 15 animals/group at each time point. BAL, bronchoalveolar lavage; CCSP(-/-), Clara cell secretory protein deficient; Mac, macrophages; PMN, polymorphonuclear neutrophils; Lym, lymphocytes. Cells in BAL were isolated by cytospin centrifugation and stained as described in MATERIALS AND METHODS. *Significantly different from wild-type mice, P ≤ 0.05.
neutrophils were detected in BAL fluid from CCSP(-/-) mice 24 h after adenoviral infection compared with wild-type control mice.

Increased cytokine and chemokine responses after adenoviral infection in CCSP(-/-) mice. Cytokine and chemokine mRNAs were assessed from lung homogenates of CCSP(-/-) and control mice 4 and 24 h after adenoviral infection. The proinflammatory cytokine TNF-α and IL-6 as well as the neutrophilic chemokine MIP-2 and MIP-1α mRNAs were unchanged 4 h after infection in either wild-type or CCSP(-/-) mice (data not shown). However, 24 h after infection, the concentration of IL-6 was increased in wild-type mice compared with uninfected control mice (Fig. 2). In CCSP(-/-) mice 24 h after infection, the concentration of IL-6 was increased threefold compared with that in wild-type mice. Concentrations of IL-1β and TNF-α were also increased to a greater extent in CCSP(-/-) mice 24 h after infection compared with wild-type mice.

Concentrations of the proinflammatory cytokines TNF-α, IL-6, and IL-1β were measured in lung homogenates from CCSP(-/-) and wild-type mice by ELISA. Four hours after infection, IL-6, IL-1β, and TNF-α were unchanged in either wild-type or CCSP(-/-) mice (data not shown). However, 24 h after infection, the concentration of IL-6 was increased in wild-type mice compared with uninfected control mice (Fig. 3). In CCSP(-/-) mice 24 h after infection, the concentration of IL-6 was increased threefold compared with that in wild-type mice. Concentrations of IL-1β and TNF-α were also increased to a greater extent in CCSP(-/-) mice 24 h after infection compared with wild-type mice.

Concentrations of the proinflammatory cytokines TNF-α, IL-6, and IL-1β were measured in lung homogenates from CCSP(-/-) and wild-type mice by ELISA. Four hours after infection, IL-6, IL-1β, and TNF-α were unchanged in either wild-type or CCSP(-/-) mice (data not shown). However, 24 h after infection, the concentration of IL-6 was increased in wild-type mice compared with uninfected control mice (Fig. 2). In CCSP(-/-) mice 24 h after infection, the concentration of IL-6 was increased threefold compared with that in wild-type mice. Concentrations of IL-1β and TNF-α were also increased to a greater extent in CCSP(-/-) mice 24 h after infection compared with wild-type mice.

Concentrations of the proinflammatory cytokines TNF-α, IL-6, and IL-1β were measured in lung homogenates from CCSP(-/-) and wild-type mice by ELISA. Four hours after infection, IL-6, IL-1β, and TNF-α were unchanged in either wild-type or CCSP(-/-) mice (data not shown). However, 24 h after infection, the concentration of IL-6 was increased in wild-type mice compared with uninfected control mice (Fig. 2). In CCSP(-/-) mice 24 h after infection, the concentration of IL-6 was increased threefold compared with that in wild-type mice. Concentrations of IL-1β and TNF-α were also increased to a greater extent in CCSP(-/-) mice 24 h after infection compared with wild-type mice.

Concentrations of the proinflammatory cytokines TNF-α, IL-6, and IL-1β were measured in lung homogenates from CCSP(-/-) and wild-type mice by ELISA. Four hours after infection, IL-6, IL-1β, and TNF-α were unchanged in either wild-type or CCSP(-/-) mice (data not shown). However, 24 h after infection, the concentration of IL-6 was increased in wild-type mice compared with uninfected control mice (Fig. 2). In CCSP(-/-) mice 24 h after infection, the concentration of IL-6 was increased threefold compared with that in wild-type mice. Concentrations of IL-1β and TNF-α were also increased to a greater extent in CCSP(-/-) mice 24 h after infection compared with wild-type mice.

Concentrations of the proinflammatory cytokines TNF-α, IL-6, and IL-1β were measured in lung homogenates from CCSP(-/-) and wild-type mice by ELISA. Four hours after infection, IL-6, IL-1β, and TNF-α were unchanged in either wild-type or CCSP(-/-) mice (data not shown). However, 24 h after infection, the concentration of IL-6 was increased in wild-type mice compared with uninfected control mice (Fig. 2). In CCSP(-/-) mice 24 h after infection, the concentration of IL-6 was increased threefold compared with that in wild-type mice. Concentrations of IL-1β and TNF-α were also increased to a greater extent in CCSP(-/-) mice 24 h after infection compared with wild-type mice.
mice compared with wild-type mice 24 h after adenoviral infection.

Adenoviral gene expression is reduced in the lungs of CCSP(−/−) mice. The adenoviral vector Av1Luc1 used in these studies encodes the luciferase reporter gene under the control of the Rous sarcoma virus promoter region. To determine whether viral gene expression is altered in the lungs of CCSP(−/−) mice after adenoviral infection, luciferase activity was measured in lung homogenates of control and CCSP(−/−) mice 7 and 14 days after adenoviral infection. Luciferase activity in control mice was higher 7 days after adenoviral infection and was decreased 14 days after infection. Luciferase activity in the lungs of CCSP(−/−) mice was significantly decreased compared with that in the lungs of wild-type mice 7 and 14 days after adenoviral administration (Fig. 4). Luciferase activity in the lungs of CCSP(−/−) mice was higher at 7 days after infection and decreased at 14 days after adenoviral administration.

Increased lung inflammation in CCSP(−/−) mice after adenoviral infection. To determine whether adenoviral-mediated lung inflammation was influenced by CCSP, lung histology was assessed after administration of the adenovirus. Pulmonary infiltrates were observed in the lungs of all mice receiving adenovirus at 7 and 14 days after infection. In wild-type mice 7 days after infection, lung inflammation consisted of focal alveolar infiltrates composed primarily of mononuclear cells, with occasional neutrophils (Fig. 5). In CCSP(−/−) mice 7 days after infection, alveolar inflammation consisted of large areas of consolidation. Lung inflammation in CCSP(−/−) mice was more extensive and involved more regions of the lung. Lung inflammation was increased 14 days after infection in both CCSP(−/−) and wild-type mice. Severe lung inflammation persisted in CCSP(−/−) mice and included alveolar septal thickening, with extensive regions of consolidation noted in the lung parenchyma. Lung inflammation in the CCSP(−/−) mice was increased compared with that in wild-type mice 7 and 14 days after administration of the virus.

DISCUSSION

The present study demonstrates increased inflammatory responses in CCSP gene-targeted mice after intratracheal administration of Av1Luc1, an E1- and E3-deleted recombinant adenoviral vector. Inflammatory cells in BAL fluid were increased in CCSP(−/−) mice, and neutrophils appeared earlier in the course of infection. Expression of the proinflammatory cytokines IL-6, IL-1β, and TNF-α as well as the neutrophilic chemokines MIP-1α and MIP-2 and the monocytic
chemokine MCP-1 were increased in the lungs of CCSP(−/−) mice after infection. Lung inflammation was increased and luciferase activity, as a measure of viral gene expression, was decreased in the lungs of CCSP(−/−) mice. These results indicate that CCSP deficiency exacerbates the early host responses and inflammation to adenoviral infection in the lung.

In a previous study (2) using the mouse model of adenoviral pneumonia, lung infiltrates appeared in the lung parenchyma 2 days after infection and were monocytic or neutrophilic in appearance. In the present study, total inflammatory cells in BAL fluid and neutrophilic infiltration increased earlier in CCSP(−/−) mice than in wild-type mice and were associated with earlier expression of cytokines and chemokines in the CCSP(−/−) mice. The induction of inflammatory responses and cytokine production after adenoviral exposure in wild-type mice is consistent with previous findings regarding host inflammatory responses after adenoviral infection (2). The findings in the present study that inflammatory cell influx and proinflammatory cytokines and chemokines are increased in the lungs of CCSP(−/−) mice are consistent with the concept that CCSP plays a role in limiting alveolar macrophages and neutrophils and cytokine responses early during the course of adenoviral infection.

Adenoviral infection is associated with acute and chronic cytopathic effects that disrupt the respiratory epithelium (2, 22). After adenoviral vector administration in mice, respiratory epithelial cell proliferation is increased in both normal and immunodeficient mice, suggesting that adenoviral infection per se has cytopathic effects independent of viral clearance by immune cells (22). Surfactant protein homeostasis was also disrupted in both immunocompetent and immunodeficient mice after adenoviral vector infection (21). In the present study, the increase in lung inflammation observed in CCSP(−/−) mice may reflect a possible role for CCSP in cytoprotection of the lung epithelium after injury. In support of this concept, CCSP(−/−) mice succumbed to lung injury earlier than wild-type mice during oxygen exposure (5).

In the present study, monocytic and neutrophilic infiltration was more extensive in the lung parenchyma of CCSP(−/−) mice after adenoviral infection. The early enhancement of cytokine expression in CCSP(−/−) mice likely contributes to the increase in lung inflammation seen later in the course of infection. The proinflammatory cytokines IL-6 and TNF-α initiate both acute and chronic inflammatory events, including the activation of adhesion molecules and chemokines (10). TNF-α also exerts important cytopathic effects on virally infected cells (8, 9). Increased expression of the chemokines MIP-1α, MIP-2, and MCP-1 in adenoviral-infected CCSP(−/−) mice may contribute to increased lung inflammation by induction of inflammatory cell chemotaxis. The findings in the present study suggest that CCSP deficiency increases the expression of important inflammatory mediators after adenoviral infection and may influence later lung inflammatory events.

Host immune responses to adenoviral vectors limit the efficiency and duration of viral gene expression. Adenoviral vector administration to immunodeficient mouse models (1, 18, 19, 22) or the use of immunomodulatory therapies (3, 22) limits host immune responses and extends the duration and level of adenoviral-mediated gene expression. The loss of adenoviral transgene expression has been attributed, at least in part, to the rapid loss of viral DNA in infected cells (22). A number of mechanisms may explain the loss of adenoviral DNA, including the cytopathic effects of virus infection, the uptake and depletion of viral particles by macrophages and other inflammatory cells, and T cell-mediated cytolytic killing during the later phase (7–14 days) of infection. In the present study, adenoviral gene expression in CCSP(−/−) mice was decreased and associated with increased pulmonary inflammation. Thus the present study is consistent with previous findings that host immune and inflammatory responses to adenoviral infection limit adenoviral vector gene expression in vivo.

Although the function of CCSP has not been clearly defined, there is increasing evidence that CCSP plays an important role in the modulation of various inflammatory responses (4, 5, 7, 11, 20). In a hyperoxic lung injury model, survival of CCSP(−/−) mice was reduced compared with control mice (5). Likewise, the onset of lung edema occurred earlier in CCSP(−/−) mice. Expression of the proinflammatory cytokines IL-3, IL-6, and IL-1β was increased in the lungs of CCSP(−/−) mice, and in the case of IL-1β, increased expression was localized to the lung parenchyma (5). Lung inflammation and injury in hyperoxic CCSP(−/−) mice were not limited to the bronchial or epithelium but also involved the alveolar epithelium, suggesting that CCSP plays a role in limiting lung injury and inflammation in both the alveolar and bronchial regions of the lung. In the present study, lung inflammation in CCSP(−/−) mice was increased markedly in the lung parenchyma compared with that in wild-type mice. Whether CCSP produced by conducting airway cells traffic to alveolar regions of the lung is not known at present. It is also possible that CCSP itself modulates inflammation in the lung parenchyma by events mediated by its action in the conducting airways.

The findings in the present study suggest that the lack of CCSP increases the host response to viral infection in the lung. Alternately, altered Clara cell function may also contribute to the observed increase in lung inflammatory responses in CCSP(−/−) mice. Ultrastructural analysis demonstrated that secretory granules in Clara cells of CCSP(−/−) mice were abnormal or absent (14). Thus it is possible that altered inflammatory responses to adenoviral infection in the present study resulted from disrupted Clara cell function rather than the lack of CCSP. Clara cells secrete a number of host defense molecules, including SP-A and SP-D (13). Both SP-A and SP-D are likely important in host defense after lung infection (17). Thus the production and secretion of important immunomodulatory
factors by the lung epithelium may play an important role in lung injury after infection.

Despite the relative abundance of CCSP in the BAL fluid, the physiological function of CCSP is not understood. CCSP (also termed CC10 and UG) has been shown in vitro to act as an immunosuppressant mediated, in part, by its ability to inhibit secretory phospholipases $A_2$. Recently, another gene-targeted mouse model of CCSP called the UG gene-targeted [UG(−/−)] mouse model was described (20). UG(−/−) mice spontaneously developed severe renal fibrosis, with extensive deposition of fibronectin and collagen. As in the present study involving the CCSP(−/−) mice, no lung pathology was reported in the UG(−/−) mouse model. The CCSP(−/−) mice used in the present study have no apparent renal pathology. The discrepancy in phenotype between the two CCSP(−/−) mice may be related to differences in vivarium conditions or genetic strains.

The present findings support the concept that CCSP functions to modulate the host responses during viral-induced lung inflammation. CCSP deficiency exacerbates early inflammatory responses to viral infection, suggesting that CCSP plays a role in innate immunity to infectious agents. Cytokines and chemokines likely play a role in the transition of early nonspecific immune responses to more specific adaptive immune mechanisms. In the present study, the enhanced induction of cytokines and chemokines in the CCSP(−/−) mice after adenoviral infection may explain the increased lung inflammation later during the course of infection and supports the concept that secretory products of lung epithelial cells are important modulators of lung inflammation and injury. The precise molecular mechanisms by which CCSP limits lung inflammation in vivo remain to be discerned. However, the present findings support the potential utility of CCSP as a therapeutic strategy to influence inflammation after lung injury and infection.

We thank Shibja Jain-Vora and Michael Jones for preparation of the cytokine primers, Nannette Mittereder and Bruce Trapnell for preparation of the adenoviral vector, and Anne Marie Levine and Thomas Kroghen for thoughtful discussion.

This work was supported by the Cystic Fibrosis Foundation; National Heart, Lung, and Blood Institute Grants HL-41496 (to J. A. Whitsett) and HL-51376 (to B. R. Stripp); and the Parker B. Francis Foundation (K. S. Harrod).

Address for reprint requests: J. A. Whitsett, Children’s Hospital Medical Center, Div. of Neonatology and Pulmonary Biology, 3333 Burnet Ave., Cincinnati, OH 45229-3039.

Received 20 March 1998; accepted in final form 14 August 1998.

REFERENCES


