Characterization of LPS-induced lung inflammation in cftr<sup>−/−</sup> mice and the effect of docosahexaenoic acid

STEVEN D. FREEDMAN,1 DEBORAH WEINSTEIN,1 PAOLA G. BLANCO,1 PEDRO MARTINEZ-CLARK,1 SERGE URMAN,1 MUNIR ZAMAN,1 JASON D. MORROW,2 AND JUAN G. ALVAREZ3

Departments of 1Medicine and 2Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215; and 3Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Received 6 September 2001; accepted in final form 9 January 2002

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations of the gene encoding the CF transmembrane conductance regulator (CFTR) (23). Patients with CF express a typical phenotype characterized by pancreatic insufficiency, ileal hypertrophy, and recurrent pulmonary infections that ultimately lead to pulmonary failure and death. Pulmonary disease in CF is characterized by excessive inflammation in response to infection by Pseudomonas aeruginosa. It has been recently reported that, in the absence of detectable lung infection, the bronchoalveolar lavage (BAL) fluid of CF patients contains increased levels of proinflammatory cytokines and neutrophils (2, 18, 20). In addition, interleukin (IL)-8 levels in BAL fluid from children with CF are significantly higher than those in non-CF children with bacterial infection of the lower airways (21). Furthermore, basal secretion of IL-6 and IL-8 was higher in human CF bronchial gland cells than in human gland cells obtained from normal individuals (16, 26). These data suggest that CFTR mutations may lead to an excessive inflammatory response in the lung.

The mechanism responsible for this enhanced inflammatory response has been difficult to study in vivo because cftr<sup>−/−</sup> mice [University of North Carolina (UNC) knockouts] show little spontaneous lung inflammation. To circumvent this problem, a model has been established whereby instillation of agarose beads coated with Pseudomonas into the lungs of S489X cftr<sup>−/−</sup> mice has been shown to result in increased inflammation and mortality compared with that observed in wild-type (WT) mice (13, 14). However, in those studies, the mortality rate was significant in both control and cftr<sup>−/−</sup> mice. This could be due, at least in part, to airway obstruction after instillation of the agarose beads.

Our laboratory (11) has recently reported the presence of a membrane lipid defect in lung, pancreas, and ileum from cftr<sup>−/−</sup> mice characterized by an increase in phospholipid-bound arachidonic acid and a decrease in phospholipid-bound docosahexaenoic acid (DHA). Correction of this fatty acid defect with high doses of oral DHA led to reversal of the pathological manifestations of CF in pancreas and ileum. However, cftr<sup>−/−</sup> mice do not express spontaneous pulmonary disease. Therefore, an animal model was established whereby mice were exposed to aerosolized Pseudomonas lipopolysaccharide (LPS) once a day for 3 days to mimic persistent infection in CF. This demonstrated a twofold increase...
in neutrophil concentration in BAL fluid from cftr−/− mice compared with that observed in WT mice (11).
The objective of this study was to determine which specific proinflammatory mediators may be responsible for the enhanced inflammatory response observed in lungs from cftr−/− mice after exposure to Pseudomonas LPS and whether oral DHA suppresses this enhanced inflammatory response. Using this model, we demonstrate that there is an increased neutrophil infiltration in lungs from cftr−/− mice compared with WT mice in response to Pseudomonas LPS and that this neutrophil infiltration is preceded by an increase in the production of tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-2 (MIP-2), and KC. In addition, the levels of the eicosanoids PGF1α, 6-keto-PGF1α, PGE2, and thromboxane B2 (TxB2) were all increased in BAL fluid from cftr−/− mice compared with those in WT controls. Oral administration of DHA resulted in a selective decrease in these eicosanoids in cftr−/− mice that was not observed in WT mice.

METHODS

Breeding of cftr−/− mice and oral administration of DHA. Experiments were approved by the Beth Israel Deaconess Medical Center Animal Care Committee. A breeding colony was established with UNC heterozygous cftr−/− exon 10 knockout mice (Jackson Laboratories, Bar Harbor, ME). Tail clip samples of 14-day-old mice were processed for analysis of genotype (28). Both WT (C57 as well as UNC cftr−/− mice) and cftr−/− mice were weaned at 23 days of age. After weaning, age-matched WT and cftr−/− mice were placed on water and Peptamen (Nestle Clinical Nutrition, Deerfield, IL) ad libitum until 30 days of age and then continued for 7 days with Peptamen or 40 mg/day of DHA (Sigma Chemical, St. Louis, MO) prepared as a stable emulsion in Peptamen. The volume of Peptamen administered was measured on a daily basis with specific feeders.

Analysis of lung inflammation. Weight-matched WT and cftr−/− mice, with and without pretreatment with oral DHA, were given a single dose of aerosolized Pseudomonas LPS (10 mg/15 g body wt, unless indicated) over 15 min once a day for up to 3 days. Pseudomonas LPS (Sigma Chemical) was sonicated in saline and administered at 20 lb/in.2 with a nebulizer connected to a compressed air tank, which was connected to a large plastic container with vent holes into which the animals were placed. It should be noted that WT mice generally weigh more than age-matched cftr−/− mice, and thus weight-matched animals were used in experiments. All animals were between 30 and 37 days of age.

Mice were killed 3, 6, or 10 h after receiving their last dose of Pseudomonas LPS. BAL was done by using four aliquots of 1 ml of sterile saline containing 1× protease inhibitor cocktail (Sigma Chemical). This was injected through polyethylene tubing placed into the upper airway after an incision into the trachea. Each aliquot of saline was introduced and removed slowly over 1 min to minimize trauma and hence red blood cell contamination. One-milliliter aliquots provided optimal lavage of the lungs without significant trauma.

Neutrophils were obtained after centrifugation of BAL fluid at room temperature for 8 min at 800 g. The resulting pellet was resuspended in 50 μl of PBS, and neutrophil concentration was determined by phase-contrast microscopy. Neutrophils were positively identified by myeloperoxidase staining and microscopic analysis by using tetramethylbenzidine-H2O2 as the peroxidase substrate. The remaining BAL fluid was centrifuged at 14,000 g for 10 min, and the supernatant was analyzed for cytokine and chemokine levels. For leukotriene B4 (LTB4) analysis, BAL fluid was centrifuged at 1,000 g for 10 min, then concentrated under reduced pressure (SpeedVac), resuspended in 0.25 ml of 50% aqueous methanol, and analyzed by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI) (19). TNF-α, MIP-2, KC, and IL-1β levels in BAL fluid were determined with commercially available EIA kits (R&D Systems, Minneapolis, MN) with lower limits of detection of 10, 5, 15, and 5 pg/ml, respectively. PGF1α, 6-keto-PGF1α, PGE2, and TxB2 were quantified by using stable isotope dilution methodology employing gas chromatography-negative ion chemical ionization mass spectrometry (7).

Statistical analysis. The differences between the means were evaluated by using Student’s t-test comparing two conditions and ANOVA for comparing three or more variables.

RESULTS

Selection of Pseudomonas LPS dose. To determine the optimum dose of LPS, aerosolized Pseudomonas LPS was administered at different doses to WT mice, and, after 3 h, neutrophil, TNF-α, and KC levels in BAL fluid were measured (Fig. 1). Neutrophils, TNF-α, and KC were undetectable in BAL fluid of mice treated with LPS-free saline. After administration of up to 5 mg LPS/15 g body wt, neutrophil concentration and TNF-α in BAL fluid increased linearly (P < 0.05 comparing 0, 1, and 5 mg LPS by ANOVA), with no further significant increase at 10 mg LPS/15 g body wt. KC levels were also increased in BAL fluid in a dose-dependent manner, with the exception that the increase was statistically different (P < 0.05) among all values examined from 0 to 10 mg LPS/15 g body wt. Because 10 mg LPS/15 g body wt resulted in maximal neutrophil levels, this dose was used for subsequent experiments. Also, because there was no significant difference in the results obtained from UNC cftr−/+ mice compared with C57 WT mice either for a single dose of aerosolized LPS or after 2 or 3 days of repeated daily administration of aerosolized Pseudomonas LPS (data not shown), the latter were used for subsequent experiments.

Repeated administration of Pseudomonas LPS. The effects of repeated administration of aerosolized Pseudomonas LPS on cytokine, chemokine, and neutrophil levels were examined in WT mice. TNF-α, MIP-2, and KC levels in BAL fluid peaked on day 1, 3 h after the initial inhalation (Fig. 2). On the next 2 days, levels of these inflammatory mediators also peaked 3 h after LPS inhalation. However, these levels were two- to threefold lower than peak levels observed on day 1. IL-1β increased after 2 days of aerosolized LPS exposure to only twice its level on day 1 with no further significant increase. In contrast, neutrophil concentration in BAL fluid increased slowly on day 1 relative to cytokine and chemokine levels, exhibiting a twofold increase from day 1 to day 2. Neutrophil concentration in BAL fluid remained relatively constant at these levels on days 2 and 3, with the occurrence of small spikes that coincided with the increase in cytokine
levels. Neutrophils were the predominant inflammatory cells with <3% lymphocytes and macrophages identifiable by light microscopy (Fig. 3A). Neutrophils were present throughout the air spaces, including both bronchi and alveoli.

Comparison of cytokine, chemokine, and neutrophil levels in cftr−/− and WT mice after Pseudomonas LPS-induced inflammation. We then investigated whether levels of these inflammatory mediators were different in cftr−/− compared with WT mice. TNF-α, MIP-2, and KC levels in BAL fluid on day 2 were significantly higher (P < 0.05) in cftr−/− mice than in WT mice (Fig. 4). IL-1β levels were low, with no significant differences between cftr−/− and WT mice. Although a similar trend was observed after 3 days of aerosolized Pseudomonas LPS, only TNF-α demonstrated a statistically significant difference (P < 0.05) in cftr−/− compared with WT mice (Fig. 5).

Eicosanoid levels in cftr−/− and WT mice after Pseudomonas LPS-induced inflammation. Eicosanoids were also examined in this model after administration of aerosolized LPS for 3 days. 6-keto-PGF1α, PGF2α, PGE2, and TxB2 levels were significantly higher in BAL fluid from cftr−/− compared with WT mice (P < 0.05) (Fig. 6A). No significant differences in LTB4 levels were observed between cftr−/− and WT mice (Fig. 6B).

Effect of DHA pretreatment on Pseudomonas LPS-induced inflammation. Pretreatment of either cftr−/− or WT mice with oral DHA had no effect on IL-1β after either 2 or 3 days of aerosolized Pseudomonas LPS exposure (Figs. 4 and 5). Similarly, DHA administration to WT or cftr−/− mice did not cause significant changes in MIP-2 or KC levels in BAL fluid after 2 and 3 days of exposure to aerosolized LPS. WT mice treated with oral DHA demonstrated a statistically significant decrease in TNF-α levels (P < 0.05) after 2 and 3 days of Pseudomonas LPS exposure compared with untreated WT mice (Figs. 4 and 5). Although similar trends in TNF-α levels were observed after DHA administration to cftr−/− mice, these changes were not statistically significant.

Eicosanoids were also examined under these conditions. Pretreatment of cftr−/− mice with oral DHA resulted in a significant decrease in 6-keto-PGF1α, PGF2α, PGE2, and TxB2 levels in BAL fluid (P < 0.05) (Fig. 6). These levels were similar to those observed in WT animals either treated or not treated with DHA. LTB4 levels were not significantly altered by DHA.

Cytokine production and neutrophil concentration in BAL fluid. To determine the relationship between cytokine levels and neutrophil infiltration in cftr−/− and WT mice, neutrophil concentration in BAL fluid was determined at different times after LPS exposure. Neutrophil concentration in BAL fluid was significantly increased in cftr−/− mice compared with WT controls after exposure to aerosolized LPS for 3 days (P = 0.004) (Fig. 7). This is seen in the representative micrographs shown in Fig. 3. This difference in neutrophil concentration between WT and cftr−/− mice was not observed on day 2. Pretreatment of cftr−/− mice with oral DHA suppressed the increase in neutrophil concentration observed on day 3 to levels found in WT mice but had no effect on neutrophil concentration on day 2. Pretreatment of WT mice with DHA did not have any significant effect on neutrophil concentration in BAL fluid from WT mice on either day 2 or 3.

DISCUSSION

Because chronic exposure to Pseudomonas plays a major role in the pathogenesis of CF-induced lung disease, we have examined the effects of repeated aerosolized Pseudomonas LPS exposure on the genesis of lung inflammation in cftr−/− mice. This model has the advantage that the mortality rate is negligible in contrast to the 23% mortality rate observed in WT mice after intratracheal instillation of Pseudomonas-coated agarose beads (14). Using a different approach, Thomas et al. (26a) have shown that intravenous administration of LPS leads to a qualitative increase in the number of neutrophils in the lung parenchyma of mice with the G551D mutation in CFTR compared

J Appl Physiol • VOL 92 • MAY 2002 • www.jap.org
with WT control mice, although the air spaces were devoid of inflammatory cells. Davidson et al. (8) examined the effects of aerosolized Staphylococcus aureus and Burkholderia cepacia given daily in low doses for up to 1 mo to cftr<sup>−/−</sup> mice. In these animals, which display a milder phenotype, a predominantly lymphoid infiltrate was present in the lungs and was associated with goblet cell hyperplasia and mucus retention. No mortality was observed. However, the pneumonia observed in CF patients is characterized by a predominantly neutrophilic infiltrate, and thus this model is not representative of lung disease in CF patients. Our present model, which results in a predominantly neutrophilic infiltrate, is more representative of lung disease in humans, although alveoli tend to be less involved in humans with CF. However, both our model as well as the Pseudomonas-coated agarose bead model result in infiltration of inflammatory cells in the upper airways as well as the alveoli (13). Although important information can be learned from LPS-stimulated inflammation, Pseudomonas factors in addition to LPS are also likely to influence the host response and progression to chronic infection in human CF, such as secreted toxins, proteases, alginate, and other virulence factors.

The fact that an increase in neutrophil concentration in BAL fluid from cftr<sup>−/−</sup> mice, compared with WT controls, was observed after 3 but not after 2 days of aerosolized Pseudomonas LPS exposure allowed us to attempt to discriminate which inflammatory mediators may be responsible for this excessive inflammatory response in cftr<sup>−/−</sup> mice. In cftr<sup>−/−</sup> mice, the increase in neutrophils was preceded by a rise in TNF-α levels, with WT control mice, although the air spaces were devoid of inflammatory cells. Davidson et al. (8) examined the effects of aerosolized Staphylococcus aureus and Burkholderia cepacia given daily in low doses for up to 1 mo to cftr<sup>−/−</sup>H11002 mice. In these animals, which display a milder phenotype, a predominantly lymphoid infiltrate was present in the lungs and was associated with goblet cell hyperplasia and mucus retention. No mortality was observed. However, the pneumonia observed in CF patients is characterized by a predominantly neutrophilic infiltrate, and thus this model is not representative of lung disease in CF patients. Our present model, which results in a predominantly neutrophilic infiltrate, is more representative of lung disease in humans, although alveoli tend to be less involved in humans with CF. However, both our model as well as the Pseudomonas-coated agarose bead model result in infiltration of inflammatory cells in the upper airways as well as the alveoli (13). Although important information can be learned from LPS-stimulated inflammation, Pseudomonas factors in addition to LPS are also likely to influence the host response and progression to chronic infection in human CF, such as secreted toxins, proteases, alginate, and other virulence factors.

The fact that an increase in neutrophil concentration in BAL fluid from cftr<sup>−/−</sup> mice, compared with WT controls, was observed after 3 but not after 2 days of aerosolized Pseudomonas LPS exposure allowed us to attempt to discriminate which inflammatory mediators may be responsible for this excessive inflammatory response in cftr<sup>−/−</sup> mice. In cftr<sup>−/−</sup> mice, the increase in neutrophils was preceded by a rise in TNF-α levels,
which were significantly increased above WT values after 2 and 3 days of aerosolized *Pseudomonas* LPS. Although KC and MIP-2 levels on days 2 and 3 were higher in *cftr*−/− mice than in WT, statistical significance was reached only on day 2. These data suggest

![Graph](http://example.com/graph1.png)

**Fig. 4.** Cytokine and chemokine levels in *cftr*−/− and WT mice exposed to aerosolized *Pseudomonas* LPS for 2 days. Weight-matched mice fed either Peptamen (*cftr*−/− and WT) or Peptamen containing 40 mg of docosahexaenoic acid (DHA) for 7 days (*cftr*−/− + DHA and WT + DHA) were exposed to aerosolized *Pseudomonas* LPS once a day for 2 days. Mice were killed 3 h after the last dose of LPS, and BAL was performed. Total levels of TNF-α, IL-1β, KC, and MIP-2 in BAL fluid are shown for each group. Values are means ± SE and correspond to at least 7 different experiments per group.

*Significant difference, *P* < 0.05.

![Graph](http://example.com/graph2.png)

**Fig. 5.** Cytokine and chemokine levels in *cftr*−/− and WT mice exposed to aerosolized *Pseudomonas* LPS for 3 days. The same conditions were utilized as per Fig. 4, except that WT and *cftr*−/− mice were exposed to LPS for 3 days. *Significant difference, *P* < 0.05.

![Graph](http://example.com/graph3.png)

**Fig. 6.** Eicosanoid levels in BAL fluid from *cftr*−/− and WT mice treated with aerosolized *Pseudomonas* LPS. Weight-matched *cftr*−/− and WT mice fed either Peptamen or Peptamen containing 40 mg of DHA were exposed to aerosolized *Pseudomonas* LPS once each day for 3 days. Mice were killed 3 h after the last dose of LPS, and BAL was performed. 

A: 6-keto-PGF₁α, PGF₂α, PGE₂, and thromboxane B₂ (TxB₂) levels were examined. B: leukotriene B₄ (LTB₄) levels were measured. Values are means ± SE from at least 3 separate experiments. *Significant difference, *P* < 0.05.

![Graph](http://example.com/graph4.png)

that TNF-α, MIP-2, and KC may be important mediators of the enhanced inflammatory response observed in lungs from *cftr*−/− mice. This is in agreement with the results obtained after intratracheal instillation of *Pseudomonas*-coated agarose beads, where these were the only inflammatory mediators that demonstrated a statistically significant increase compared with WT
controls (14). However, in that model, neutrophil levels were minimally increased in cftr−/− compared with WT mice, whereas LTBA and IL-1β levels were markedly increased, although these differences were not statistically significant. In our model, LTBA levels were not different between cftr−/− and WT mice under these conditions. Pretreatment of cftr−/− mice with oral DHA did not significantly decrease TNF-α, MIP-2, or KC levels. Similarly, DHA administration did not significantly alter MIP-2 and KC levels in WT mice, although TNF-α levels were statistically decreased. Taken together, these data indicate that, under the conditions utilized, DHA treatment does not suppress these proinflammatory cytokines in the lungs of cftr−/− mice.

Eicosanoids were also examined to determine their role in this inflammatory process. 6-Keto-PGF1α, PGF2α, PGE2, and TxB2 levels were elevated in cftr−/− mice compared with WT control animals, suggesting that these arachidonic acid metabolites may also play an important role in the initial stages of the enhanced lung inflammatory response observed in cftr−/− mice. This is in agreement with data from other groups demonstrating an increase in eicosanoids in BAL fluid (19), saliva (22a), and urine (24a) from CF patients. The fact that pretreatment of cftr−/− mice, but not WT mice, with oral DHA resulted in suppression of these eicosanoids suggests that DHA may be reversing a specific abnormality related to CFTR dysfunction, perhaps related to the abnormality in fatty acid metabolism. Specifically, the elevated levels of phospholipid-bound arachidonic acid may be directly responsible for the increased production of eicosanoids. Administration of oral DHA, by competing for incorporation at the sn-2 position in membrane phospholipids, decreases arachidonic acid levels, thereby lowering the production of eicosanoids. However, the lowering of eicosanoids is not a generalized phenomena, because LTBA levels were not affected by oral administration of DHA in this animal model. Because virtually all cells can synthesize eicosanoids, it is difficult to identify which cell type(s) is responsible for the increased levels produced in the lungs of cftr−/− mice. Outside of LTBA, there are few studies examining whether other eicosanoids participate in leukocyte recruitment. Of the four eicosanoids found to be increased in this model of aerosolized LPS-induced lung inflammation and specifically suppressed by oral DHA, only PGF2α has been shown to have potent polymorphonuclear neutrophil chemotactic activity (1, 15, 24). This was not observed with PGE2 (1), and 6-keto-PGF1α, and TxB2 have not been directly tested. These data suggest that the decrease in neutrophil concentration in BAL fluid after pretreatment of cftr−/− mice with DHA may be, in part, mediated through a decrease in PGF2α-induced neutrophil recruitment. Further experiments that use specific inhibitors of PGF2α may clarify the role of this molecule.

There is increasing evidence that a defect in CFTR function leads to an exaggerated inflammatory host response in both respiratory epithelial cells, as well as in lung resident macrophages. This appears to be a constitutive abnormality, because CF bronchial submucosal glands cultured from CF patients demonstrate an increase in basal IL-6 and IL-8 levels compared with non-CF control cells (16, 26). This increase in IL-8 levels can be explained by significant amounts of constitutively activated nuclear factor-κB (NF-κB) in these CF cell lines (9). In addition, human CF bronchial gland cells display a lack of cytosolic factor IκBa, also resulting in an upregulation of IL-8 production compared with non-CF disease bronchial gland cells (25). These results were confirmed in cultured human CF bronchial gland cells in which a lack of cytosolic IκBa and high levels of constitutively activated NF-κB, associated with an upregulation of IL-8 production, were found compared with non-CF disease bronchial gland cells. In addition to this increase in these proinflammatory mediators, IL-10 levels have been reported to be markedly decreased both in BAL fluid (6) and in isolated bronchial epithelial cells from CF patients (5). Furthermore, the notion that CFTR mutations predispose to enhanced lung inflammation is supported by the fact that BAL fluid from CF infants, before demonstrable colonization or infection occurs, contains higher concentrations of neutrophils and IL-8 compared with BAL fluid from disease control infants (2, 18).

In an effort to determine whether inflammatory cells in addition to respiratory epithelial cells are involved in the genesis of this altered inflammatory response, macrophages from CF patients, both differentiated in vitro from monocytes (22) and from BAL fluid (6, 18), have been shown to secrete increased levels of IL-8 as well as TNF-α. This is in agreement with the fact that
monocytes contain low levels of CFTR based on RT-PCR and southern analysis (27). In the present model, it should be emphasized that respiratory epithelial cells, in contrast to the macrophage, minimally respond to bacterial LPS with no stimulation of IL-8 secretion (10). Therefore, the initial inflammatory response to LPS is generated by the lung macrophage. Production of TNF-α and other cytokines by macrophages after exposure to Pseudomonas LPS may lead to secondary production of cytokines by respiratory epithelial cells and to an amplification of the inflammatory response. Whether the increase in TNF-α, MIP-2, and KC levels in BAL fluid from cftr−/− mice of observed exposure to Pseudomonas LPS is of macrophage origin, from the respiratory epithelium, or a combination cannot be ascertained in this model. An alternative mechanism that would explain our findings of an exaggerated inflammatory response in CF is a defect in the development of tolerance to repeated LPS exposure. Endotoxin tolerance was first described by Beeson (3), who observed a decrease in febrile response after repeated injection of rabbits with endotoxin, and appears to be a protective mechanism to prevent uncontrolled immunological activation in the host after septicemia that could lead to continuous production of proinflammatory cytokines, such as TNF-α, and to severe vascular collapse (29). The principal cells responsible for the development of this tolerance effect are monocytes and macrophages (12). This effect is not secondary to a decrease in CD14, the LPS receptor on the cell surface of these cells, but perhaps is due to a formation of inactive NF-κB complexes consisting of p50 homodimers (4). Alternatively, tolerance may result from the downregulation of IL-12 secretion observed after chronic exposure of human monocytes to LPS in culture (17). Taken together, these data would be consistent with the notion that the development of endotoxin tolerance is defective in CF and perhaps DHA modulates this tolerance mechanism. Further experiments are required to determine the validity of this hypothesis in cftr−/− mice.

Our previous data indicate that a membrane lipid imbalance is present in ileum, pancreas, and lung from cftr−/− mice, characterized by a selective increase in phospholipid-bound arachidonic acid and a decrease in phospholipid-bound DHA (11). Treatment of these mice with oral DHA resulted in correction of the lipid abnormality as well as reversal of the pathology in pancreas and ileum. Although the effects of DHA on suppression of TNF, MIP-2, and KC levels in BAL fluid were similar in both WT and cftr−/− mice exposed to Pseudomonas LPS, the fact that neutrophils and the eicosanoids studied were decreased only in cftr−/− mice but not WT animals suggests that the membrane lipid imbalance observed in lungs from cftr−/− mice may play an important role in the pathogenesis of this enhanced pulmonary inflammation.

We thank Dr. Rene Mora from the Department of Medicine at Beth Israel Deaconess Medical Center for help in setting up the Pseudomonas LPS nebulizer apparatus. We also thank Dr. Christoph Karp from the Department of Infectious Diseases at The Johns Hopkins University for critical review of this manuscript.

This work was supported by the Cystic Fibrosis Foundation (J. G. Alvarez and S. D. Freedman) and by National Institutes of Health Grants GM-15431, DK-48831, and CA-77839 (to J. D. Morrow). J. D. Morrow is also the recipient of a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research.

REFERENCES


