

Acute *Pseudomonas* challenge in cystic fibrosis mice causes prolonged nuclear factor- κ B activation, cytokine secretion, and persistent lung inflammation

Aicha Saadane, PhD, Jindrich Soltys, DVM, PhD, and Melvin Berger, MD, PhD

Cleveland, Ohio

Background: Cystic fibrosis (CF) is characterized by an excessive and prolonged inflammatory response to *Pseudomonas aeruginosa* in the lung. There are high levels of cytokines and chemokines and an exaggerated PMN influx causing significant morbidity and mortality.

Objective: To compare the kinetics of the inflammatory response with the kinetics of clearance of acute bacterial challenge in the lungs of CF and wild-type (WT) mice.

Methods: We challenged CF knockout (KO) and WT mice intratracheally with *P aeruginosa* in suspension and evaluated bacteria counts, nuclear factor- κ B (NF- κ B), and inhibitor of NF- κ B alpha protein (I- κ B α) in lung tissue, cytokines, and PMN in bronchoalveolar lavage (BAL).

Results: Both groups of mice cleared the infection with the same kinetics. CF-KO mice had more PMN in BAL than WT mice. CF-KO mice had high concentrations of proinflammatory cytokines in BAL on days 2 and 4, whereas cytokines in BAL from WT mice were only slightly elevated. CF-KO mice failed to regenerate I- κ B α once it was degraded, and consequently had prolonged and excessive activation of NF- κ B for the entire 6-day duration of the study. In contrast, WT mice showed only slight NF- κ B activation, which plateaued at day 4.

Conclusion: These data suggest that NF- κ B is dysregulated in CF lung infection and could be a good target for therapy. Prolonged responses to initial acute infections may contribute to the eventual establishment of chronic persistent inflammation.

Clinical implications: Dysregulation of the I- κ B/NF- κ B pathway in cystic fibrosis leads to prolonged cytokine secretion and persistent inflammation in response to acute challenges and may be important in the development of chronic lung inflammation and infection. (J Allergy Clin Immunol 2006;117:1163-9.)

Key words: Cystic fibrosis, bronchoalveolar lavage, *Pseudomonas aeruginosa*, nuclear factor- κ B, I- κ B, lung, PMN

Abbreviations used

BAL:	Bronchoalveolar lavage
CF:	Cystic fibrosis
CFTR:	Cystic fibrosis transmembrane regulator
CFU:	Colony-forming units
EMSA:	Electrophoretic mobility shift assay
I- κ B:	Inhibitor of NF- κ B
KO:	Knockout
MIP:	Macrophage inflammatory protein
NF- κ B:	Nuclear factor- κ B
WT:	Wild-type

Most of the morbidity and almost all of the mortality in cystic fibrosis (CF) is caused by an apparently excessive inflammatory response to chronic infection of the airways with pathogens including *Pseudomonas aeruginosa*. Studies of bronchial lavage (BAL) fluid and cells isolated from CF patients suggest that the immune and inflammatory responses in the CF lung are disproportionate to the threat posed by infection.¹⁻⁵ Patients with CF have higher IL-8 and PMN in their BAL than patients without CF with similar burdens of bacteria or LPS in their BAL.^{4,5} Moreover, babies with CF may have increased BAL PMN and IL-8 even in the absence of demonstrable infection.^{1,4,6} We have previously presented data suggesting that these babies may have been transiently infected and then had prolonged inflammatory responses that persisted after transient infection was cleared.¹ We have recently shown that CF knockout (KO) mice have exaggerated and prolonged inflammatory responses after acute intratracheal challenge with LPS.⁷ Our goal in this study was to determine whether there are similarly prolonged responses to acute bacterial challenge, and to study the signaling pathways involved.

Many proinflammatory cytokines that are greatly elevated in BAL of patients with CF^{1,2,4,5} and animal models of CF lung disease^{1,2,4,5,7-12} require nuclear factor- κ B (NF- κ B) for maximal transcription. Cultured epithelial cells with defects in cystic fibrosis transmembrane regulator (CFTR), compared with cells with normal CFTR, have greater activation of NF- κ B in response to multiple stimuli.^{3,8-10,14,15} NF- κ B normally resides in the cytoplasm in complex with inhibitor of NF- κ B (I- κ B).¹⁶ In response to stimuli, I- κ B is rapidly phosphorylated by the I- κ B

From the Department of Pediatrics, Rainbow Babies and Childrens' Hospital, Case Western Reserve University School of Medicine.

Supported by grants from the Cystic Fibrosis Foundation.

Disclosure of potential conflict of interest: The authors have received grant support from the Cystic Fibrosis Foundation.

Received for publication August 29, 2005; revised January 9, 2006; accepted for publication January 12, 2006.

Reprint requests: Melvin Berger, MD, PhD, Department of Pediatrics, Case Western Reserve University, 11100 Euclid Avenue, Cleveland, OH 44106. E-mail: mxb12@po.cwru.edu.

0091-6749/\$32.00

© 2006 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2006.01.052

kinase complex.¹⁶ This allows liberation of NF- κ B from I- κ B and its translocation to the nucleus, where it promotes proinflammatory gene expression.

Until now, there have been few studies of the kinetics and the signaling pathways involved in the inflammatory response to infection in the intact CF lung. In this study, we used an acute infection model to evaluate the kinetics of transient lung infection in CF mice that would mimic the situation in transiently infected babies with CF.¹ The results show that acute *P aeruginosa* infection in CF-KO mice leads to a prolonged and excessive inflammatory response, with increased PMN influx and higher concentrations of proinflammatory cytokines and chemokines in BAL than in wild-type (WT) mice given the same inoculum. In contrast, there were no differences in the rate of clearance of bacteria from the lung, suggesting that differences in the regulation of inflammation rather than differences in the efficiency of host defense mechanisms are responsible for the increased and prolonged PMN influx into the lung. We found that the lungs of the CF-KO mice had increased and persistent activation of NF- κ B. This in turn was associated with increased consumption and/or impaired replacement of I- κ B α .

METHODS

Mice

Male CF-KO mice 7 to 10 weeks old homozygous for the S489X (B6.129P2-Cftr^{tm1Unc}) (null) mutation¹⁷ and normal wt/wt (WT) littermates were used after genotyping by polymerase chain reaction on tail snip DNA. Pups were weaned at 10 days of life and fed Peptamen (Clintech Nutrition Co, Deerfield, Ill) to avoid intestinal obstruction.¹⁸ All mice were maintained in sterile microisolator cages with sterile water.

Experimental model

To generate a transient acute infection, 82 CF-KO and 93 WT mice were anesthetized then inoculated into the trachea with a plastic catheter angled toward the right mainstream bronchus^{19,20} with 3 to 4 $\times 10^6$ colony-forming units (CFU) *P aeruginosa*, strain M5715 (a clinical isolate), suspended in 20 μ L PBS.

Mice were randomized at the time of inoculation as to which day after inoculation they would be killed (day 2, 4, or 6) and then randomly assigned to 1 of 2 outcome measures: removal and homogenization of lungs for quantitative bacteriology, or BAL for analysis of inflammatory cells and mediators, followed by homogenization. Lung homogenates from the latter group were frozen, then later used for Western blot analysis for I- κ B α and electrophoretic mobility shift assays (EMSAs) for activated NF- κ B. Four uninfected CF-KO and 7 uninfected WT mice were also killed to determine baseline parameters.

Quantitative bacteriology

Quantitative bacteriology was determined by plating serial dilutions of whole lung homogenates.^{19,20} To determine whether there had been dissemination of bacteria from the lung resulting in bacteremia, spleens from the same mice were homogenized and cultured.

BAL fluid analysis

Bronchoalveolar lavage was performed as previously described.²⁰ TNF- α , IL-1 β , IL-6, KC, and macrophage inflammatory protein (MIP)-2 were assayed by using commercial ELISA kits (R&D

Systems, Minneapolis, Minn) by a technician unaware of the origin of the samples.

Western blot analysis for I- κ B α

After BAL, the lung vasculature was flushed with ice-cold sterile PBS. Lungs were then removed and homogenized.²¹ SDS-polyacrylamide gels and Western blots were used to detect I- κ B α as previously described.²⁰ Blots were also probed with monoclonal anti- β -actin to correct for protein loading.

EMSA

Nuclear extracts of whole lung tissues were prepared,⁷ and EMSA was performed with γ [³²P] ATP (Amersham Pharmacia Biotech) end-labeled double-stranded NF- κ B consensus sequence probe (Promega, Madison, Wis). To confirm the specificity of binding of ³²P-labeled NF- κ B probe, we added unlabeled NF- κ B or activator protein-1 (AP-1) consensus oligonucleotides as competitors in the same assay (Promega).

Statistical analysis

Two-factor ANOVAs were used with mouse groups and days for percent PMNs, total BAL cell counts, and bacteria clearance. For cytokine data, the minimum detectable limit was assigned to values below the limit of detection of the ELISA assays. Data are expressed as means $\pm$ SEMs.

RESULTS

In this acute model, *P aeruginosa* is suspended evenly in sterile PBS. Preliminary experiments were conducted to determine the appropriate inoculum that would allow us to study both the clearance of bacteria and the kinetics of the inflammatory response without causing early mortality. Results of these experiments showed that doses of bacteria in the range of 5 $\times 10^6$ CFU in 20 μ L led to more than 90% mortality in both CF-KO and WT mice. With lower inocula, survival was better. In the studies reported here, mice were challenged intratracheally with 3.5 to 4 $\times 10^6$ CFU in 20 μ L suspension, then killed at 2-day intervals over a period of 6 days. Of 82 CF-KO mice inoculated with *P aeruginosa*, 20 died before their designated day of sacrifice (75.6% survival), and of 93 WT mice inoculated with *P aeruginosa*, 14 died before their designated time of sacrifice (84.9% survival). This difference in mortality was not statistically significant ($P = .15$). Preliminary data showed that female CF-KO mice had higher mortality with these inocula than male CF-KO mice. Thus, only male mice were used.

Quantitative bacteriology

To determine whether there was any difference between CF-KO and WT in the clearance of bacteria from their lungs, quantitative bacteriology was performed on lung homogenates from mice killed on different days after *P aeruginosa* challenge. As shown in Fig 1, there was no difference in the ability of CF-KO and WT mice to clear bacteria. Both types of mice reduced the density of bacteria by more than 4 logs by the first time point, 2 days after inoculation. The same low density of bacteria, less than 0.01% of the inoculum, remained present in both types of mice through the end of the experiment on day 6. When the

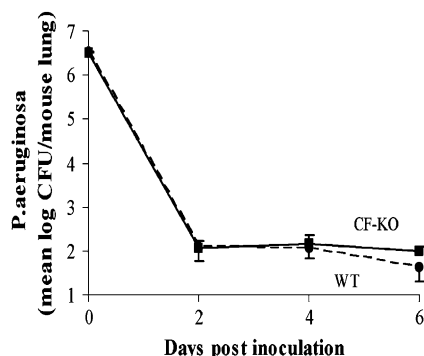


FIG 1. Quantitative bacteriology in CF-KO (squares, $n = 11$) and WT (circles, $n = 13$) mice inoculated with *P aeruginosa*. Values represent the mean $\pm$ SEMs of $\log_{10}$ of CFU per mouse lung 2, 4, and 6 days after inoculation. The CFUs at day 0 indicate the inoculating dose.

$\log_{10}$ CFU/mouse lung was compared for each individual time point, there were no significant differences between CF-KO and WT mice (day 2, $P = .98$; day 4, $P = .23$; day 6, $P = .44$). There was no evidence of dissemination of infection, as determined by culture of spleen homogenates, in either group of mice.

BAL fluid cell counts and differentials

Twenty-four infected CF-KO mice and 27 infected WT mice were randomized to undergo BAL. Eleven additional uninfected mice, 4 CF-KO and 7 WT, were killed to determine baseline BAL fluid cell counts and differentials on day 0. There were few PMNs in the BAL of uninfected mice (Fig 2, day 0) with no significant difference between CF-KO and WT mice. The total number of leukocytes in BAL fluid ($\log_{10}$ leukocytes/mL BAL) from both groups was higher 2 days after inoculation than at baseline (6.28 ± 0.24 and 5.79 ± 0.24 , respectively, in CF-KO and WT mice). By day 4, CF-KO mice had significantly more total leukocytes than WT mice (6.16 ± 0.31 vs 5.59 ± 0.24 ; $P = .05$). After that, both types of mice showed a decrease toward the baseline (data not shown). The percentage of PMNs is shown in Fig 2. This peaked at day 2 postinoculation in CF-KO as well as WT mice. The peak PMN response was slightly higher in CF-KO than in WT mice ($67.11 \pm 5.6\%$ vs $58.5 \pm 11.6\%$, respectively; $P = .3$). However, the decrease toward baseline after day 2 was more rapid in WT mice. On day 4, CF-KO mice also had a significantly greater percentage of PMNs in BAL fluid compared with WT mice ($46.9 \pm 7.4\%$ vs $18.5 \pm 2.2\%$, respectively; $P = .004$). Similar results were seen for the absolute number of PMNs: CF-KO mice had higher PMNs than WT mice (mean $\log_{10}$ PMNs/mL BAL) by day 2 (6.10 ± 0.23 vs 5.57 ± 0.44) and significantly more PMNs at day 4 (5.92 ± 0.18 vs 5.16 ± 0.21 ; $P = .01$; data not shown). Note that this corresponds to a time point at which the bacterial density was quite low, and not different in the 2 types of mice (Fig 1).

BAL fluid cytokine concentrations

We next sought to determine whether the differences in PMN might be a result of differences in concentrations

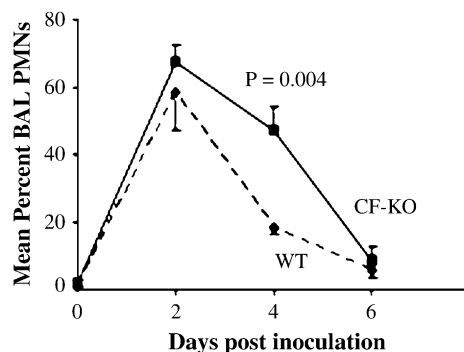


FIG 2. Percent PMNs in BAL fluid in CF-KO (squares, $n = 26$) and WT (circles, $n = 25$) mice inoculated with *P aeruginosa*. Results shown are means $\pm$ SEMs of the percent PMNs in BAL fluid 2, 4, and 6 days after inoculation. The 0 time point is for unchallenged controls.

in BAL of the proinflammatory cytokines TNF- α and/or IL-1 β , and IL-6, as well as the chemokines KC and MIP-2, whose function is similar to IL-8 in human beings. Baseline concentrations of all of these mediators in BAL fluid from uninfected controls of both types were below the limits of detection (Fig 3). However, they became significantly more elevated in BAL fluid from inoculated CF-KO mice than in inoculated WT controls. Consistent with its role as an important early-response cytokine, TNF- α peaked at day 2 after inoculation, but it was much higher in the CF-KO mice and barely elevated in the WT mice (214.9 ± 43.7 vs 37.7 ± 21.7 , respectively, in CF-KO and WT mice; $P = .04$; Fig 3, A). TNF- α remained elevated at day 4 in the CF-KO mice, whereas in WT mice, it had already returned to baseline. This increase in TNF was associated with a trend toward more profound loss of weight in CF-KO than WT mice ($-11.14\% \pm 1.22\%$ vs $-7.87\% \pm 2.12\%$, $P = .21$, respectively in CF-KO and WT mice). Weight loss was most pronounced on day 2 postinoculation, after which both types of mice gradually started regaining weight. However, CF-KO regained their body weight slower than WT mice ($-10.18\% \pm 2.36\%$ vs $-2.45\% \pm 1.32\%$, $P = .02$ at day 4, and $-5.76\% \pm 5.29\%$ vs $-2.25\% \pm 1.41\%$, $P = .44$, at day 6, respectively, in CF-KO and WT mice).

Results for IL-1 β were similarly elevated in CF-KO compared with WT, but the IL-1 β peaked later than the TNF, at day 4 (Fig 3, B). IL-6 also peaked on day 4 and was significantly higher in CF-KO vs WT mice (Fig 3, C). This was also true for the neutrophil chemoattractant MIP-2, which peaked on day 4 as well and was significantly different from WT mice (Fig 3, D). Most mice had levels of KC that were under the detection limit of the assay (3.9 pg/mL) except 2 CF-KO mice out of 7 that had 27.46 and 34.7 pg/mL KC at day 4 and 1 WT mouse out of 6 that had 17.9 pg/mL at day 6.

Activation of NF- κ B in lung tissue of CF-KO mice challenged with *P aeruginosa*

The excessive and prolonged inflammatory response in CF-KO mice, with high concentrations of proinflammatory cytokines and chemokines whose genes depend on

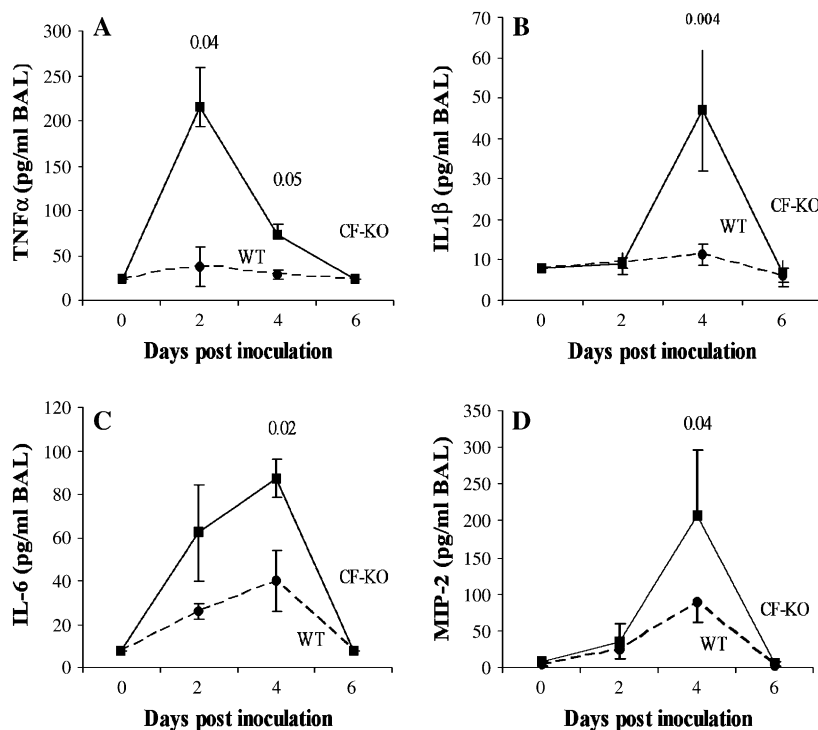


FIG 3. BAL fluid proinflammatory cytokines in CF-KO (squares, $n = 26$) and WT (circles, $n = 25$) mice inoculated with *P aeruginosa*. Means $\pm$ SEMs of concentrations in BAL fluid 2, 4, and 6 days after inoculation.

NF- κ B for maximal transcription, suggested that activation of that transcription factor might be excessive and/or prolonged. We therefore examined the kinetics of NF- κ B activation with EMSA of tissue from perfused lungs from mice that had undergone BAL on days 2, 4, and 6 postinoculation. Because BAL removed inflammatory cells from the lumen of the airways and from the airspaces, and the perfusion removed intravascular leukocytes, the remaining tissue should be predominately parenchymal lung cells. No activated NF- κ B was detected in uninoculated mice of either type (Fig 4, day 0). Activated NF- κ B was strongly present in tissue obtained from CF-KO mice 2 days after the challenge, whereas in WT mice, only a weak signal was seen at this time (Fig 4, A). NF- κ B activation continued in CF-KO mice and was greater in CF-KO mice than in WT mice at all time points after inoculation (Fig 4, B). The specificity of NF- κ B binding was confirmed by using excess unlabeled NF- κ B and AP-1 oligonucleotides (Fig 4, C).

Changes in lung content of I- κ B α

We sought to determine whether the increased and prolonged activation of NF- κ B we observed after challenge of the CF-KO mice might be a result of excessive degradation and/or deficient replacement of I- κ B α . Western blots show that baseline expression of I- κ B α was not significantly different in uninfected CF-KO vs WT mice (Fig 5, B, day 0: 19 ± 2 vs 29 ± 5 ; $P = .19$). In both KO and WT mice, the I- κ B α was reduced at 2 days after inoculation (Fig 5). However, by day 4, the WT

mice had replaced the I- κ B α , which was at even higher concentrations than the baseline. In contrast, in the CF-KO mice, the I- κ B α remained depressed for the entire 6-day course of the experiment. I- κ B α was significantly reduced in CF-KO compared with WT mice on days 4 and 6 ($P = .0006$ and $.001$, respectively).

DISCUSSION

Cystic fibrosis is the most common genetic disease causing early death in white patients. Although the pathway from gene defect to clinical manifestations is not yet completely defined, increasing evidence suggests that a dysregulated and excessive inflammatory response to infection with *P aeruginosa* is what actually kills the patients. However, to date, there have been no studies of the kinetics of NF- κ B activation in response to acute *P aeruginosa* challenge of the lungs of intact CF compared with non-CF animals. To test the hypothesis that dysregulation of NF- κ B signaling leads to the dysregulated inflammatory response to infection in CF lungs, we challenged CF-KO and WT mice intratracheally with *P aeruginosa* in suspension. Unlike chronic models using agar beads that hold the bacteria in the airway and markedly decrease clearance of the infection, resulting in chronic inflammation,²² in this acute model, *P aeruginosa* is suspended in PBS, and almost all of the organisms (99.99%) are killed within the first few days after inoculation. Thus, the inciting stimulus is greatly reduced. Therefore, we

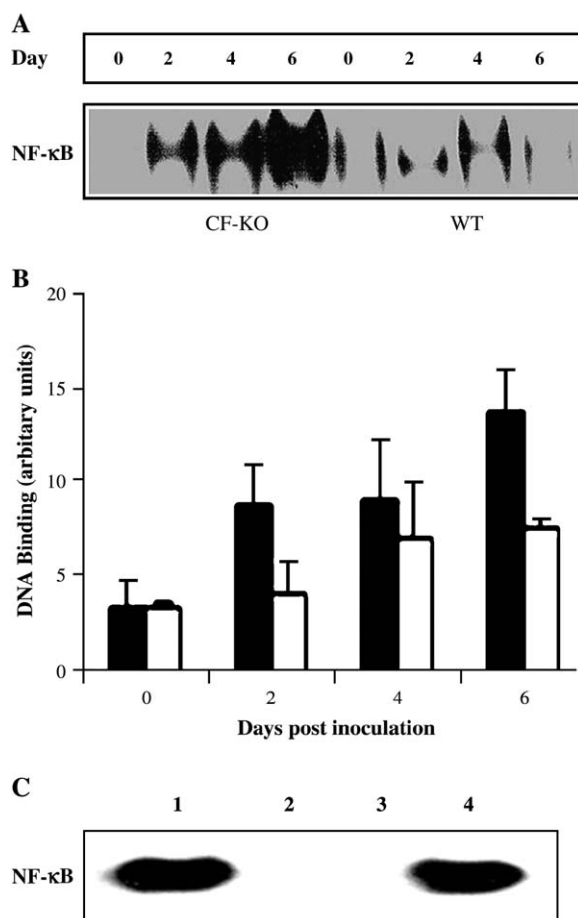


FIG 4. A, Representative EMSA for activated NF-κB in homogenates of perfused whole lungs from CF-KO and WT mice that had undergone BAL and were killed 2, 4, and 6 days after *P aeruginosa*. **B**, The amount of complexed NF-κB probe estimated by image scanning and expressed in arbitrary units; CF-KO, closed bars, and WT mice, open bars. Three separate experiments are included. Results shown are means $\pm$ SEMs for 3 to 4 mice for each bar. **C**, Controls for specificity of EMSA: lane 1, labeled probe with extract known to contain active NF-κB; lane 2, labeled probe with no extract; lane 3, labeled probe with extract plus excess of unlabeled NF-κB oligonucleotide; lane 4, labeled probe with extract plus excess of unlabeled AP-1 oligonucleotide.

could study the kinetics of the resolution of the inflammatory response once the stimulus for that response has been essentially removed.

Our data show that the lungs of CF-KO mice after intratracheal inoculation with *P aeruginosa* in suspension share many characteristics with the pulmonary disease in patients with CF. Like patients with CF, BAL from inoculated CF-KO mice contains higher numbers of neutrophils and elevated concentrations of proinflammatory mediators and chemokines compared with WT mice. However, both groups of mice exhibited similar kinetics of bacterial clearance. This suggests that the excessive inflammation may be a result of dysregulation of the inflammatory response in CF-KO mice rather than a difference in their ability to eradicate the bacteria. This is an agreement

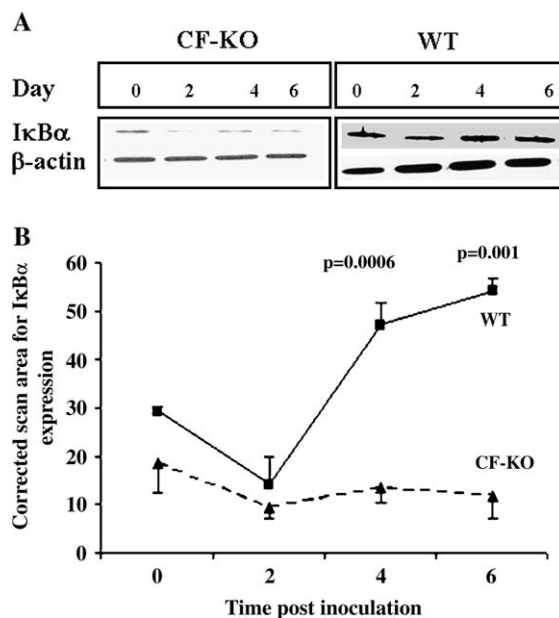


FIG 5. A, Western blots showing time course of IκBα content of homogenate of whole lung from CF-KO versus WT mice inoculated with *P aeruginosa*. Equal protein application was verified by equal staining for β-actin. Representative of 3 separate experiments. **B**, Densitometric scan area of total IκBα on Western blots corrected by normalization to scan area of β-actin. Results are average values of 3 independent experiments.

with our former data, which showed an excessive and prolonged inflammatory response to intratracheal LPS in CF-KO compared with WT mice.⁷ Despite the same small burden of bacteria remaining in the lungs of both groups of mice on day 4, CF-KO mice had significantly more BAL PMNs persisting at that time than WT mice. The cytokine data suggest that at least part of the persistence of the PMNs in BAL fluid was a result of the excessive and prolonged production of TNF, IL-1β, and the PMN chemoattractant MIP-2. It is of interest to note that, consistent with its role as an important early response cytokine, TNF-α peaked at 2 days after *P aeruginosa* challenge, whereas the other proinflammatory cytokines, IL-1β and IL-6, peaked later, at 4 days postinoculation. Also, the chemokine MIP-2 peaked later, at day 4. This is when the greatest difference in PMN was seen.

The TNF-α peaked in CF-KO mice at 300 pg/mL, whereas in WT controls, the peak TNF was less than 80 pg/mL. Because both CF-KO and WT mice cleared the infection with the same kinetics, this suggests that the small amount of TNF and the more modest PMN response in the WT mice were sufficient to control the bacteria. Therefore, the huge increase in TNF in the CF-KO mice and the exuberant and prolonged PMN may be considered to be in excess of what is required for adequate host defense. This is in accord with studies showing high levels of TNF in BAL in patients with CF.² Interestingly, the timing of the most severe weight loss (day 2) corresponds with the most dramatic difference in TNF-α concentration.

Similarly, cachexia in patients with CF might be a result of excessive concentrations of TNF.²³ Hence, we view TNF as a pivotal proinflammatory cytokine that plays an important role in inducing the excessive chronic inflammatory response in the CF lung and additional systemic effects as well. CF-KO mice treated intratracheally with LPS also showed a similarly exaggerated TNF response.⁷ Freedman et al¹⁰ showed that repeated aerosolized LPS administration leads to increased concentrations of TNF and CXC chemokines in BAL from CF-KO compared with WT control mice. Those authors found differences between CF-KO and WT mice only after repeated exposures to aerosolized LPS.¹⁰ We speculate that reinoculating mice that are already overresponding, like those here on the 4th day, might result in even greater differences between CF and WT. Two other studies, using mice with the G551D mutation in CFTR, also showed that those mice display an abnormal inflammatory response with increased cytokines and infiltrating cells.^{24,25}

Because the genes for proinflammatory cytokines, such as TNF, IL-1 β , and IL-6, are activated by NF- κ B,²² we studied NF- κ B activation in the lung tissue. Consistent with the high numbers of PMNs and elevated BAL concentrations of cytokines on day 4, CF-KO mice showed excessive and prolonged activation of NF- κ B compared with WT mice. This continued in CF-KO mice as long as 6 days postinoculation. However, by this time, the results for PMNs and cytokines had returned to baseline. NF- κ B is not likely to be the sole transcription factor involved in upregulating these cytokines, and these discrepancies between NF- κ B and the other measures might be an indication of the involvement of other transcription factors in the net cytokine production. These data provide an example of how biologically relevant stimuli, such as bacteria, can modulate proinflammatory signal transduction pathways differently from what would be predicted by the effects of isolated signals, such as LPS.⁷ Thus, the prolonged activation of NF- κ B could explain at least in part the excessive and prolonged inflammatory response to infection in CF-KO mice. Our results are consistent with results of *in vitro* studies that demonstrate excessive activation of NF- κ B in primary cultures of CF airway epithelial cells^{3,14} and in cell lines with CF defects.^{8,9,13,15} Because we lavaged the lungs extensively, 6 times with 0.5 mL PBS each time, and the pulmonary circulation was flushed until the lungs were totally blanched, we believe that the excessive NF- κ B activation we observed primarily reflects the status of lung parenchymal cells rather than infiltrating inflammatory cells. Two recent studies have addressed the fundamental role of airway epithelial cell NF- κ B activation in inhaled LPS models, using transgenic mice in which NF- κ B activation was selectively prevented in airway epithelial cells.^{26,27} This significantly blunted local inflammatory responses. We previously reported that airway epithelial cells removed from infants with CF after they had cleared transient infection continued to produce IL-8 *in vitro*, whereas cells from normal controls did not.¹ Thus, the excessive and prolonged cytokine secretion and prolonged activation of NF- κ B we

observed is most likely an important site of dysregulation of proinflammatory signaling in the CF lung.

Because NF- κ B activation follows phosphorylation and degradation of its inhibitor, I- κ B, we also studied the amount of I- κ B α in extracts of lavaged, perfused lungs from these mice. There was a slight but not significant difference in I- κ B α between KO and WT mice at time 0. I- κ B α was markedly reduced 2 days after inoculation in both groups. Interestingly, whereas I- κ B α recovered to supranormal levels in WT mice 4 and 6 days after inoculation, it remained at low levels in CF-KO mice for the entire course of the experiment. These data suggest that the prolonged decrease in I- κ B α is a result of prolonged excessive activation of I- κ B kinase and/or impaired replacement synthesis of I- κ B α . Therefore, we hypothesize that the dysregulation of I- κ B α , which should normally stop the ongoing proinflammatory signaling, is likely responsible for the persistent response to transient infection we observed. We have proposed elsewhere that increased degradation and/or decreased synthesis of I- κ B α may be a consequence of local deficiency of the anti-inflammatory cytokine IL-10 in the CF lung, which may be related to the defect in CFTR.^{7,20,28,29} The prolonged cytokine production and PMN influx into the lungs of CF-KO mice after intratracheal LPS challenge were corrected by exogenous IL-10, which increased the I- κ B and decreased the activation of NF- κ B.⁷ Similarly, Tabary et al¹² recently showed that IL-10 treatment *in vitro* inhibited NF- κ B activation in human CF bronchial epithelial cells and that this was associated with reduction of I- κ B α phosphorylation and I- κ B kinase α/β expression.

The use of this acute infection model allowed us to study the kinetics of local antibacterial defense mechanisms simultaneously with the kinetics of inflammatory response. The results suggest that the dysregulation of the inflammatory response to acute infection in CF-KO mice is similar to what we have observed after transient infection in babies with CF. Because a large body of data (see review³⁰) suggests that persistence of PMN products can impair local phagocytic defense mechanisms, the type of persistent response we observed in the CF-KO mice on the 4th day after inoculation may actually set the stage for establishment of chronic, uneradicatable infection in patients with CF. The protracted inflammatory response we observed may be a result of the inability of CF-KO mice to regenerate I- κ B α once it has been degraded during the initial response to infection, and/or to downregulate I- κ kinase. In turn, this allows excessive and prolonged activation of NF- κ B, which likely contributes to increased cytokine and chemokine production and increased PMN influx seen in CF-KO mice. Therefore, pharmacologic agents that inhibit NF- κ B activation and/or function might have important therapeutic effects in CF, particularly if used early in the disease.

We thank Alma Gente Wilson, Anna van Heeckeren, and other members of the Animal Core of the Case Western Reserve University Cystic Fibrosis Center for providing us with mice. We also thank Sophia Masters for expert technical assistance.

REFERENCES

- Bonfield TL, Konstan MW, Berger M. Altered respiratory epithelial cell cytokine production in cystic fibrosis. *J Allergy Clin Immunol* 1999;104:72-8.
- Bonfield TL, Panuska JR, Konstan MW, Hilliard KA, Hilliard JB, Ghnaim H, et al. Inflammatory cytokines in cystic fibrosis lungs. *Am J Respir Crit Care Med* 1995;152:2111-8.
- Joseph T, Look D, Ferkol T. NF- κ B activation and sustained IL-8 gene expression in primary cultures of cystic fibrosis airway epithelial cells stimulated with *Pseudomonas aeruginosa*. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L471-9.
- Muhlebach MS, Noah TL. Endotoxin activity and inflammatory markers in the airways of young patients with cystic fibrosis. *Am J Respir Crit Care Med* 2002;165:911-5.
- Muhlebach MS, Stewart PW, Leigh MW, Noah TL. Quantitation of inflammatory responses to bacteria in young cystic fibrosis and control patients. *Am J Respir Crit Care Med* 1999;160:186-91.
- Balough K, McCubbin M, Weinberger M, Smits W, Ahrens R, Fick R. The relationship between infection and inflammation in the early stages of lung disease from cystic fibrosis. *Pediatr Pulmonol* 1995;20:63-70.
- Saadane A, Soltys J, Berger M. Role of IL-10 deficiency in excessive nuclear factor- κ B activation and lung inflammation in cystic fibrosis transmembrane conductance regulator knockout mice. *J Allergy Clin Immunol* 2005;115:405-11.
- Li J, Johnson XAD, Iazovovskaia S, Tan A, Lin A, Hershenov MB. Signaling intermediates required for NF- κ B activation and IL-8 expression in CF bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L307-15.
- Weber AJ, Soong G, Bryan R, Saba S, Prince A. Activation of NF- κ B in airway epithelial cells is dependent on CFTR trafficking and Cl⁻ channel function. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L71-8.
- Freedman SD, Weinstein D, Blanco PG, Martinez-Clark P, Urman S, Zaman M, et al. Characterization of LPS-induced lung inflammation in *cfr*^{-/-} mice and the effect of docosahexaenoic acid. *J Appl Physiol* 2002;92:2169-76.
- Tabary O, Escotte S, Couetil JP, Hubert D, Dusser D, Puchelle E, et al. Relationship between IkappaBalpha deficiency, NFkappaB activity and interleukin-8 production in CF human airway epithelial cells. *Pflugs Arch* 2001;443(suppl 1):S40-4.
- Tabary O, Muselet C, Escotte S, Antonicelli F, Hubert D, Dusser D, et al. Interleukin-10 inhibits elevated chemokine interleukin-8 and regulated on activation normal T cell expressed and secreted production in cystic fibrosis bronchial epithelial cells by targeting the I(k)B kinase alpha/beta complex. *Am J Pathol* 2003;162:293-302.
- DiMango E, Ratner AJ, Bryan R, Tabibi S, Prince A. Activation of NF- κ B by adherent *Pseudomonas aeruginosa* in normal and cystic fibrosis respiratory epithelial cells. *J Clin Invest* 1998;101:2598-605.
- Knorre A, Wagner M, Schaefer HE, Colledge WH, Pahl HL. DeltaF508-CFTR causes constitutive NF- κ B activation through an ER-overload response in cystic fibrosis lungs. *Biol Chem* 2002;383:271-82.
- Venkatakrishnan A, Stecenko AA, King G, Blackwell TR, Brigham KL, Christman JW, et al. Exaggerated activation of nuclear factor- κ B and altered IkappaB-beta processing in cystic fibrosis bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2000;23:396-403.
- Christman JW, Sadikot RT, Blackwell TS. The role of nuclear factor- κ B in pulmonary diseases. *Chest* 2000;117:1482-7.
- Snouwaert JN, Brigrman KK, Latour AM, Malouf NN, Boucher RC, Smithies O, et al. An animal model for cystic fibrosis made by gene targeting. *Science* 1992;257:1083-8.
- Eckman EA, Cotton CU, Kube DM, Davis PB. Dietary changes improve survival of CFTR S489X homozygous mutant mouse. *Am J Physiol Lung Cell Mol Physiol* 1995;269:L625-30.
- Chmiel JF, Konstan MW, Berger M. Murine models of CF airway inflammation. In: Skach WR, editor. *Methods in molecular medicine: cystic fibrosis methods and protocols*. Vol 70. Totowa (NJ): Humana Press, Inc; 2002. p. 495-515.
- Chmiel JF, Konstan MW, Saadane A, Krenicky JE, Kirchner HL, Berger M. Prolonged inflammatory response to acute *Pseudomonas* challenge in interleukin-10 knockout mice. *Am J Respir Crit Care Med* 2002;165:1176-81.
- Lentsch AB, Shanley TP, Sarma V, Ward PA. In vivo suppression of NF- κ B and preservation of Ikba by interleukin-10 and interleukin-13. *J Clin Invest* 1997;100:2443-8.
- van Heeckeren AM, Tscheikuna J, Walenga RW, Konstan MW, Davis PB, Erokku B, et al. Effects of *Pseudomonas* infection on weight loss, lung mechanics, and cytokines in mice. *Am J Respir Crit Care Med* 2000;161:271-9.
- Elborn JS, Cordon SM, Western PJ, Macdonald IA, Shale DJ. Tumor necrosis factor- α , resting energy expenditure and cachexia in cystic fibrosis. *Clin Sci (Lond)* 1993;85:563-8.
- Thomas GR, Costelloe EA, Lunn DP, Stacey KJ, Delaney SJ, Passey R, et al. G551D Cystic fibrosis mice exhibit abnormal regulation of inflammation in lungs and macrophages. *J Immunol* 2000;164:3870-7.
- McMorran BJ, Palmer JS, Lunn DP, Oceandy D, Costelloe EO, Thomas GR, et al. G551D CF mice display an abnormal host response and have impaired clearance of *Pseudomonas* lung disease. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L740-7.
- Poynter ME, Irvin CG, Janssen-Heininger YMW. A prominent role for airway epithelial NF- κ B activation in lipopolysaccharide-induced airway inflammation. *J Immunol* 2003;170:6257-65.
- Skerrett SJ, Liggitt HD, Hajjar AM, Ernst RK, Miller SI, Wilson CB. Respiratory epithelial cells regulate lung inflammation in response to inhaled endotoxin. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L143-52.
- Bonfield TL, Konstan MW, Burfeind P, Panuska JR, Hilliard JB, Berger M. Normal bronchial epithelial cells constitutively produce the anti-inflammatory cytokine interleukin-10, which is downregulated in cystic fibrosis. *Am J Respir Cell Mol Biol* 1995;13:257-61.
- Soltys J, Bonfield T, Chmiel J, Berger M. Functional IL-10 deficiency in the lung of cystic fibrosis (cystic fibrosis transmembrane conductance regulator ^{-/-}) and IL-10 knockout mice causes increased expression and function of B7 costimulatory molecules on alveolar macrophages. *J Immunol* 2002;168:1903-10.
- Berger M, Konstan M. Immunopathogenesis of cystic fibrosis lung disease. In: Yankaskas J, Knowles M, editors. *Cystic fibrosis in adults*. Philadelphia (PA): Lippincott-Raven; 1999. pp. 115-43.