

Role of IL-10 deficiency in excessive nuclear factor- κ B activation and lung inflammation in cystic fibrosis transmembrane conductance regulator knockout mice

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Background: Patients with cystic fibrosis (CF) and CF transmembrane conductance regulator knockout (CF-KO) mice are deficient in pulmonary IL-10 and have excessive inflammatory response to *Pseudomonas aeruginosa* infection. **Objective:** We hypothesized that local IL-10 deficiency in the lung was responsible for prolonged and excessive inflammatory responses and observations of inflammation in the absence of infection.

Methods: To determine whether IL-10 deficiency could account for persistent inflammation in CF mice independent of interactions of bacteria with epithelial cells, we challenged IL-10-knockout (IL-10-KO), CF-KO, and wild-type (WT) mice intratracheally with LPS and determined the effects of IL-10 replacement in CF-KO mice.

Results: In response to LPS, IL-10-KO and CF-KO mice had more neutrophils and proinflammatory cytokines in bronchoalveolar lavage than WT mice. Both types of knockout mice had more profound and prolonged consumption of I- κ B and increased activation of nuclear factor κ B (NF- κ B). Activated NF- κ B persisted for 6 to 8 hours in CF-KO and IL-10-KO mice but was not detected beyond 2 hours in WT mice. IL-10 treatment of CF-KO mice attenuated the reduction in I- κ B α and activation of NF- κ B and reduced the excessive inflammation.

Conclusion: Similarities in the responses of CF-KO and IL-10-KO mice and correction of excessive responses in CF mice by exogenous IL-10 suggest that deficiency of IL-10 may be responsible for prolonged and excessive inflammatory responses in CF. Because LPS was used as the stimulus, these excessive responses are independent of any possible differences in the interactions of bacteria with CF epithelial cells. (J Allergy Clin Immunol 2005;115:405-11.)

Key words: Cystic fibrosis, neutrophils, cytokines, chemokines, IL-10, NF- κ B, I- κ B

Abbreviations used

BAL:	Bronchoalveolar lavage
CF:	Cystic fibrosis
CF-KO:	Cystic fibrosis transmembrane conductance regulator knockout
CFTR:	Cystic fibrosis transmembrane conductance regulator
IL-10-KO:	IL-10-knockout
KC:	KC/N51
MIP-2:	Macrophage inflammatory protein 2
NF- κ B:	Nuclear factor κ B
WT:	Wild-type

Chronic pulmonary infection and PMN-dominated inflammation are the major causes of loss of lung function and mortality of patients with cystic fibrosis (CF). Pro-inflammatory cytokine concentrations are much higher in CF versus control bronchoalveolar lavage (BAL) fluid¹⁻⁴ and persist after transient infection is cleared.² BAL fluid from healthy controls contains IL-10, which is reduced in patients with CF^{2,5} and in CF transmembrane conductance regulator (CFTR) knockout (CF-KO) mice, even in the absence of infection.⁶ We previously demonstrated the importance of IL-10 in regulation of the inflammatory response in chronic and acute endobronchial *Pseudomonas aeruginosa* infection in mice⁷ and proposed that decreased IL-10 may contribute to the excessive inflammatory response in CF lungs.⁴ Like IL-10-knockout (IL-10-KO) mice, CF-KO mice overreact to *P aeruginosa* with higher mortality, greater and persistent cytokines and chemokines, and increased PMN in BAL.^{7,8} To distinguish between dysregulation of the inflammatory response per se versus differences in interactions of *P aeruginosa* with host epithelial cells as the cause of these increased inflammatory responses, we used intratracheal LPS to test the hypothesis that IL-10 deficiency leads to dysregulation of inflammation in the lungs of CF-KO mice. Similar abnormalities were observed in IL-10-KO and CF-KO compared with wild-type (WT) mice and were reversed by IL-10 treatment.

Many proinflammatory cytokines that can be suppressed by IL-10 and that are greatly elevated in CF patients' BAL^{3,4,9,10} are regulated by nuclear factor κ B (NF- κ B). NF- κ B normally resides in the cytoplasm in

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Supported by grants from the Cystic Fibrosis Foundation, National Institutes of Health immunology training award 645-1045, and VEGA grant 2/4179/04 to Dr Soltys.

Received for publication January 15, 2004; revised September 30, 2004; accepted for publication October 11, 2004.

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0091-6749/\$30.00

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doi:10.1016/j.jaci.2004.10.044

complex with I- κ B.¹¹ In response to stimuli, I- κ B is rapidly phosphorylated and degraded.¹² The liberated NF- κ B promotes proinflammatory gene transcription. Our results suggest that IL-10 deficiency in CF-KO and IL-10-KO mice leads to impaired replacement and/or continued consumption of I- κ B after transient stimulation, allowing increased and prolonged NF- κ B activation and dysregulation of the inflammatory response. All of these differences between CF-KO and WT mice can be corrected by IL-10 treatment of CF-KO mice.

METHODS

Mice

Male IL-10-KO mice 7 to 10 weeks old weighing approximately 25 g and age-matched, sex-matched, and weight-matched WT mice on the same background (C57Bl/6J; Jackson Laboratories, Bar Harbor, Me) were used. CF-KO mice homozygous for the S489X (B6.129P2-Cftr^{tm1Unc}; null) mutation¹³ and WT littermates were fed Peptamen (Clintech Nutrition Co, Deerfield, Ill) to avoid intestinal obstruction¹⁴ and were maintained in sterile microisolator cages. Genotypes were confirmed by PCR on tail snip DNA. All IL-10-KO mice and their WT controls were studied at less than 10 weeks of age, before inflammatory bowel disease develops.⁷

Experimental model

Mice were anesthetized and intubated through a small incision in the trachea with a plastic catheter and challenged with 100 ng LPS (*Escherichia coli* serotype 0111:B4 from Sigma, St Louis, Mo) in 50 μ L PBS.⁷

IL-10-KO (n = 77) and WT (n = 54); and CF-KO (n = 30) and WT (n = 41) mice were randomized in advance to be sacrificed 2, 4, 6, 8, or 10 hours after intratracheal challenge. At that time, mice were anesthetized and had BAL,⁷ and lungs were perfused with ice-cold saline and removed. Lungs were homogenized for Western blots for I- κ B α and extracted for NF- κ B.¹⁵ In addition, unchallenged controls underwent BAL for baseline cell counts and cytokine concentrations.

In addition, 3 CF-KO mice received 2.5 μ g recombinant mouse IL-10 (R&D Systems, Minneapolis, Minn) in 200 μ L Tris-buffered saline containing 50 μ g BSA, and 3 CF-KO mice received 200 μ L Tris-BSA alone (placebo) subcutaneously 1 hour before LPS. All protocols were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University.

BAL fluid analysis

Bronchoalveolar lavage was performed and processed as previously described.⁷ TNF- α , IL-1 β , IL-6, KC/N51 (KC), and macrophage inflammatory protein 2 (MIP-2) were assayed by ELISA (R&D Systems) by a technician unaware of the origin of the samples.

Western blot analysis for I- κ B α in lung tissue

After BAL, the pulmonary vasculature was flushed with ice-cold sterile PBS. Lungs were then removed and homogenized.¹⁵ SDS-polyacrylamide gels and Western blots were used to detect I- κ B α as previously described.⁷ Blots were also probed with monoclonal anti- β -actin to correct for protein loading.

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared from the same lung tissue used for EMSA¹⁶ with a γ [³²P]ATP labeled NF- κ B consensus sequence probe

(Promega; Madison, WI). Polyclonal rabbit anti-p65 (Santa Cruz Biotech, Santa Cruz, Calif), unlabeled NF- κ B probes, and AP-1 consensus sequence probes (Promega, Madison, Wis) were used for supershift and competition assays.

Statistical analysis

Two-factor ANOVAs were used for percent PMNs and total BAL cell counts. For cytokine data, the minimum detectable limit was assigned to values below the limit of detection in the ELISAs, and the Wilcoxon rank-sum test was used. Data are expressed as means $\pm$ SEMs.

RESULTS

Altered airway secretions and/or altered interactions of CF bronchial epithelial cells with *P aeruginosa*, activation of intracellular signaling by misfolded CFTR, and dysregulation of cytokine production have all been proposed as explanations for excessive inflammation in the airways of patients with CF and CF-KO mice.^{17,18} We previously reported that BAL from uninfected CF-KO (S489X) mice on a mixed background lacked IL-10.⁶ We are currently using S489X CF-KO mice that have been made congenic by backcrossing with C57Bl6 mice for 12 generations. We again found that even uninfected CF-KO mice have significantly less IL-10 in BAL than WT littermate controls (59 ± 3.8 vs 86 ± 8.3 in [n = 4] CF-KO and [n = 6] WT, respectively; $P = .039$). To test the hypothesis that this decrease in IL-10 contributes to excessive inflammation in the airways of CF-KO mice, independent of possible differences in interactions of bacteria with airway cells, we administered isolated LPS (*E coli* 0111:B4) intratracheally. We compared CF-KO mice with IL-10-KO mice and studied the effects of IL-10 treatment of the CF-KO mice.

Lack of inflammation in unchallenged CF-KO and WT control mice

All mice were in sterile microisolator cages and had irradiated food and endotoxin-free water. Lung homogenates were prepared from sentinel mice to be sure these precautions prevented spontaneous infection, and all cultures were negative. In BAL from all unchallenged CF-KO (n = 4) and unchallenged WT (n = 6) mice, cultures were negative, and TNF- α was below the limit of detection (23.4 pg/mL), except in 1 WT mouse (24.98 pg/mL). Neither CF-KO nor WT mice had any appreciable PMN in their BAL in the absence of LPS challenge (Fig 1, A; time = 0). Because cultures were negative, TNF was not detectable, and PMNs were minimal and not different, these data suggest that decreased IL-10 is an intrinsic property of the CF lung and that there is no spontaneous inflammation in unchallenged CF-KO or WT mice.

Cellular response to intratracheal LPS challenge

After LPS challenge, CF-KO mice had higher numbers and percentages of PMNs at all time points compared with

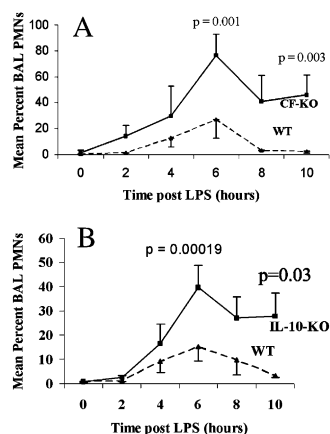


FIG 1. Percent PMNs in BAL fluid from (A) CF-KO vs WT mice and (B) IL-10-KO and WT mice. Mice were challenged intratracheally with 100 ng LPS in 50 μ L LPS. Results shown are means $\pm$ SEMs % PMN in BAL 2, 4, 6, 8, and 10 hours after LPS administration. The 0 time point is for unchallenged controls.

WT mice (Fig 1, A). PMNs peaked at 6 hours after LPS in both groups of mice but were much higher in CF-KO than in WT (77% $\pm$ 17% vs 28% $\pm$ 14%, respectively; $P < .001$). PMNs were still elevated in CF-KO mice at 8 and 10 hours after LPS administration, when few PMNs were detectable in WT mice. Thus, CF-KO mice in response to LPS are similar to patients with CF in that PMNs are the predominant inflammatory cells in BAL.¹⁹

IL-10-KO ($n = 77$) and WT ($n = 54$) mice were also challenged with LPS intratracheally. Additional unchallenged mice ($n = 7$) in each group served as controls. Neither IL-10-KO nor WT mice had any appreciable PMNs in BAL in the absence of LPS challenge (Fig 1, B; time = 0), suggesting that, as in the CF-KO mice, the deficiency of this cytokine does not lead to spontaneous pulmonary inflammation. Similarly to LPS-challenged CF-KO mice, LPS-challenged IL-10-KO mice had greater PMNs in BAL than WT mice at all points from 4 to 10 hours, which was highly significant at the peak, 6 hours after LPS ($P = .0019$; Fig 1, B). As in the CF-KO mice, PMNs were still greatly elevated at 8 and 10 hours in the IL-10-KO mice, by which time PMNs had returned to baseline in WT mice. Thus, the PMN response was also excessive, and its kinetics were quite similar in the CF-KO and IL-10-KO mice.

BAL fluid cytokine concentrations

To help understand why the PMN influx was so much greater in CF-KO versus WT mice, BAL concentrations of TNF- α , IL-1 β , and IL-6; and KC and MIP-2 (homologues to IL-8 in human beings) were assayed. None of these were detectable in BAL from any unchallenged mice except for 1 IL-10-KO out of 7, which had detectable TNF; and 1 CF-WT mouse out of 4, which gave a signal for IL-6 lower than the lowest standard.

Consistent with its role as an important early-response cytokine, TNF- α peaked at 2 hours after LPS challenge in

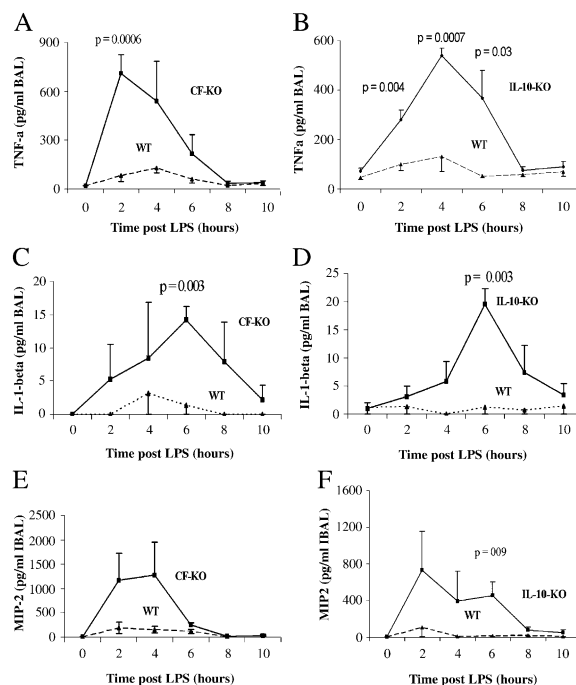


FIG 2. TNF- α , IL-1 β , and MIP-2 concentrations in BAL fluid from CF-KO vs WT mice (A, C, E) and IL-10-KO vs WT mice (B, D, F). Means $\pm$ SEMs of concentrations in BAL fluid 2, 4, 6, 8, and 10 hours after LPS administration are shown.

both CF-KO and WT mice, but it was much higher in the former (Fig 2, A; 710 $\pm$ 113 vs 83 $\pm$ 38, respectively, in CF-KO and WT mice; $P = .0006$). In CF-KO mice, TNF- α remained elevated at 4 and 6 hours, whereas in WT mice, it was only slightly increased 4 hours after LPS and dropped nearly to baseline by 6 hours. IL-10-KO mice also had significantly higher TNF- α concentrations in BAL at 2, 4, and 6 hours after LPS challenge compared with WT controls. TNF- α peaked 4 hours after LPS in IL-10-KO mice (Fig 2, B; 502 $\pm$ 176 vs 93 $\pm$ 30 for WT; $P < .015$ in IL-10-KO and WT mice, respectively) and remained significantly higher at 6 hours as well (Fig 2, B). IL-1 β was similarly elevated in both types of KO compared with WT, but IL-1 β peaked later than TNF, at 6 hours (Fig 2, C and D). Results for IL-6 were similar to those for IL-1 β in both types of KO mice, peaking at 4 to 6 hours (not shown).

Bronchoalveolar lavage from both CF-KO and IL-10-KO mice had significantly more of the neutrophil chemoattractants MIP-2 (Fig 2, E and F) and KC (not shown) at 2 through 6 hours after LPS. In contrast, WT mice had only modest increases in these chemokines, in accord with their lower PMN responses.

Activation of NF- κ B in lung tissue of IL-10-KO and CF-KO mice challenged with LPS

The high concentrations of proinflammatory cytokines and chemokines whose genes depend on NF- κ B for maximal transcription suggested activation of that transcription factor might be excessive and/or prolonged. We

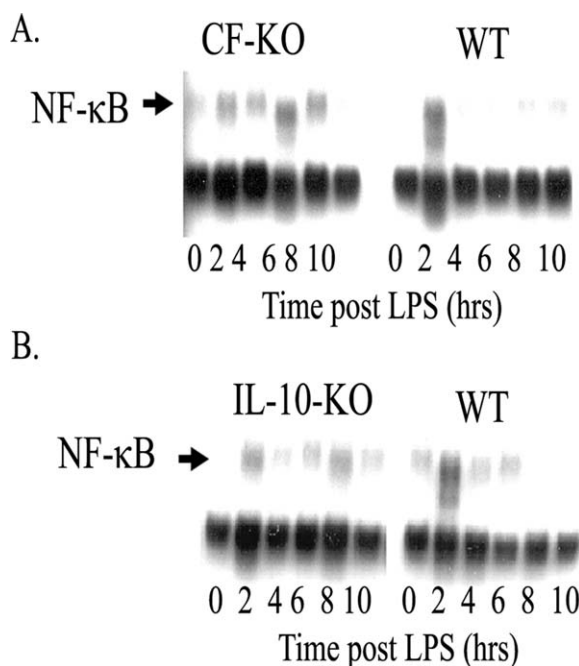


FIG 3. EMSA showing time course of NF- κ B activation after LPS (**A**) in CF-KO vs WT mice and (**B**) in IL-10-KO vs WT mice. Results are representative of 3 separate experiments.

analyzed activated NF- κ B with EMSA on lungs extracted after BAL to remove inflammatory cells from the airways and airspaces and perfusion to remove intravascular leukocytes. Thus, these extracts should predominately sample parenchymal lung cells. In CF-KO as well as WT mice challenged with LPS, NF- κ B activation was detected within 2 hours (Fig 3, A). However, although NF- κ B activation was not detected beyond this time in WT mice, it was still dramatically elevated in CF-KO mice for as long as 8 hours. The specificity of the EMSA was confirmed by using excess unlabeled NF- κ B and AP-1 oligonucleotides. Supershift assays identified p65 as a constituent of the major form of NF- κ B detected in all types of mice used in these studies (not shown).

IL-10-KO mice and controls also showed increased nuclear NF- κ B within 2 hours (Fig 3, B). Only slight activation of NF- κ B was detected beyond 2 hours in WT controls, although it was still dramatically elevated at 6 and 8 hours in IL-10-KO mice, just as it was in CF-KO mice.

Changes in lung content of I- κ B α

We used Western blots to determine whether the increased and prolonged activation of NF- κ B might be a result of excessive degradation and/or deficient replacement of I- κ B α . There was little difference in the baseline I- κ B in lung tissue of unchallenged CF-KO versus WT mice (Fig 4, A; time = 0). However, by 4 hours after LPS, there was a profound decrease in the I- κ B α in CF-KO mice, which took 10 hours to recover to baseline (Fig 4, A and B). In contrast, the WT mice already showed increased

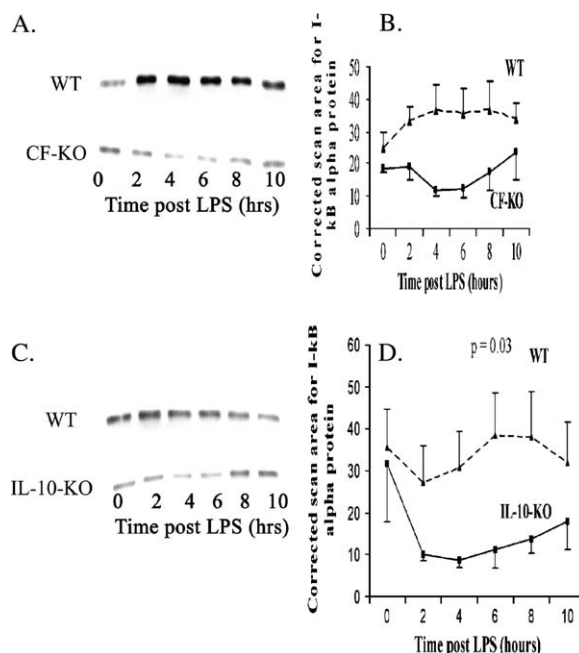


FIG 4. A, Western blots showing time course of I- κ B α content of homogenate of whole lung from CF-KO vs WT mice after LPS challenge. Representative of 4 separate experiments. B, Densitometric scan area of total I- κ B α on Western blot corrected by normalization to scan area of β -actin. C and D, Same analyses in IL-10-KO vs WT mice. Values represent means $\pm$ SEMs.

I- κ B α by 2 hours after LPS challenge. This overshoot the baseline by 4 hours and remained elevated for 10 hours.

As shown in Fig 4, C and D, IL-10-KO and WT mice also had similar levels of I- κ B α at baseline (time = 0), consistent with their lack of inflammation in the unchallenged state (Fig 1, B). Two hours after LPS, there was a marked decrease in the I- κ B α in IL-10-KO mice, whereas WT mice showed only a slight reduction. Similarly to the CF-KO mice, I- κ B α decreased by 4 and 6 hours in IL-10-KO mice, and it had not returned to baseline by 10 hours after the challenge. In contrast, in WT mice, I- κ B α was only slightly reduced at 2 and 4 hours. I- κ B α increased slightly over its baseline at 6 hours and returned to the prechallenge level by 10 hours.

In contrast with I- κ B α , I- κ B β did not differ in CF-KO versus WT controls (not shown).

IL-10 treatment attenuates excessive inflammation and NF- κ B activation and increases I- κ B α in CF-KO mice challenged with LPS

Having shown that IL-10 deficiency was associated with similarly excessive and prolonged inflammatory responses in IL-10-KO and CF-KO mice, we sought to determine whether exogenous IL-10 could reverse the differences between CF-KO and WT mice. CF-KO mice were treated with recombinant mouse IL-10 or placebo. All mice were challenged with LPS 1 hour later, then

killed 6 hours later. Although the response to LPS in this cohort of mice is not as dramatic as that shown in Fig 1, A, IL-10-treated CF-KO mice had significantly decreased numbers of PMNs (Fig 5, A) and TNF- α (Fig 5, B) in BAL compared with the placebo group. This was associated with decreased NF- κ B activation (Fig 5, C) and increased I- κ B α (Fig 5, D).

DISCUSSION

Chronic lung infection with *P aeruginosa* and excessive inflammation is the major cause of morbidity and mortality in CF. Pulmonary secretions from patients with CF contain high concentrations of the proinflammatory cytokines TNF- α , IL-1 β , IL-6, and IL-8,^{1,3,4,9} but the content of the anti-inflammatory cytokine IL-10 is reduced.^{1,2,5,9} We have shown previously⁶ and again in this study that BAL from uninfected CF-KO (S489X) mice contains less IL-10 than BAL from WT controls. Previous studies on fresh bronchial epithelial cells from normal human beings,² human airway epithelial cell lines,⁵ and normal mice²⁰ all suggest that epithelial cells are an important source of IL-10 in the normal, uninfected lung. IL-10-KO mice, like CF-KO mice, have excessive inflammation, weight loss, and mortality from chronic as well as acute lung infection with *P aeruginosa*.⁷ We postulate that IL-10 deficiency in the CF lung is a result of the CF defect and may contribute to an imbalance between proinflammatory and anti-inflammatory mechanisms, allowing the persistent and excessive inflammatory responses that characterize CF. Others have proposed that excessive inflammation in the CF lung may be a result of a decreased ability to clear or kill *P aeruginosa*,²¹ or may be a cellular response to misfolded CFTR protein.¹⁷ We hypothesized that a defect in regulating cytokine production could account for excessive inflammation independent of alterations in the host's interactions with bacteria.

In these studies, we used intratracheal LPS as a challenge, which would be independent of alterations in clearance of bacteria, to test the hypothesis that IL-10 deficiency could account for the dysregulation of the inflammatory response seen in CF. Both CF-KO and IL-10-KO mice had prolonged and excessive inflammatory responses to intratracheal LPS, with increased PMNs in the airways, mimicking BAL of patients with CF. BAL from LPS-challenged CF-KO as well as IL-10-KO mice had greatly elevated concentrations of the early proinflammatory cytokine TNF- α , as well as increased concentrations of IL-1 β and IL-6 and chemoattractants KC and MIP-2. Intriguingly, these excessive PMN and cytokine responses to LPS reflect reports that patients with CF had greater PMNs and IL-8 in BAL than patients without CF with the same concentrations of LPS in their BAL.³ Others have also shown that CF mice have greater responses to LPS than WT mice.^{22,23}

All of our mice were maintained in microisolator cages to minimize the risk of unintended infection. We found no

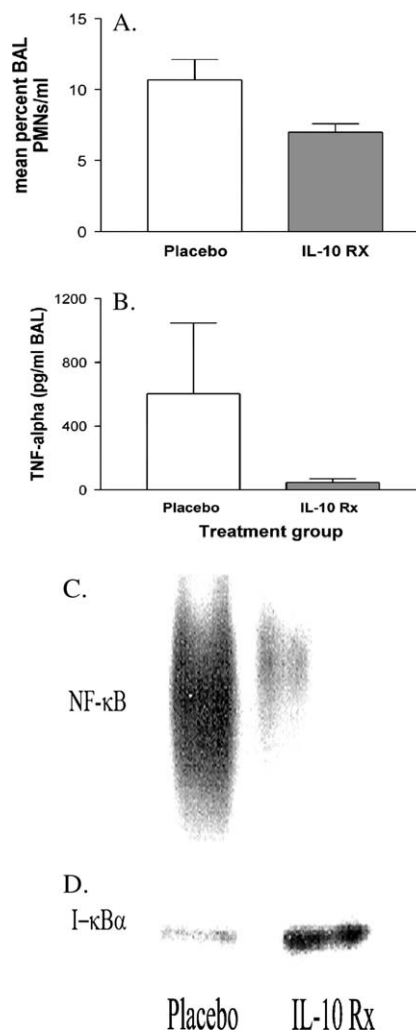


FIG 5. Effects of IL-10 treatment: CF-KO mice were injected with 2.5 μ g rmlIL-10 or with buffer alone 1 hour before LPS challenge, then killed 6 hours later. PMNs (A) and TNF- α (B) in BAL and nuclear NF- κ B (C) and total I- κ B α protein (D) in lung tissue of placebo (left) vs IL-10-treated (right) CF-KO mice. Values for PMNs and TNF- α are means $\pm$ SEMs. Representative gels are shown for NF- κ B EMSA and I- κ B α protein Western blot.

spontaneous inflammation in the lungs of CF-KO or IL-10-KO mice. However, there was much less IL-10 in the BAL of unchallenged CF-KO mice. This suggests that the decreased IL-10 may be an intrinsic defect in CF lung. Furthermore, treatment with exogenous IL-10 reduced the excessive responses in the CF-KO mice. As discussed below, CF-KO and IL-10-KO mice also had similar perturbations in the I- κ B/NF- κ B signaling cascade that were also corrected by IL-10 treatment of the CF-KO mice. Thus, it seems likely that local IL-10 deficiency in the lung contributes to, or is responsible for, the dysregulated responses observed in CF-KO mice.

Several mechanisms have been proposed for the anti-inflammatory effects of IL-10.^{15,24,25} In this acute challenge model, effects of IL-10 on T_H2 versus T_H1

differentiation of lymphocytes or effects on antigen presentation are not likely to be important. Anti-inflammatory effects of IL-10 may include suppressing production of cytokines, chemokines, and adhesion molecules that depend on NF- κ B.^{6,24,25} Recently, we demonstrated that the inflammatory response to acute, transient *P aeruginosa* infection in IL-10-KO mice is characterized by excessive proinflammatory cytokine production, which persisted after the bacteria were eradicated.⁷ Similarly prolonged production of proinflammatory cytokines after clearance of transient infection has also been reported in babies with CF,² and could account for reports of inflammation in the absence of infection.² After acute LPS challenge, both CF-KO and IL-10-KO mice had prolonged elevations of PMNs in BAL. These prolonged PMN and cytokine responses to a single intratracheal administration of LPS are consistent with an important role for IL-10 in terminating acute inflammatory responses and support the hypothesis that prolonged as well as excessive inflammation may be a result of local IL-10 deficiency.

The mechanisms of the excessive inflammatory responses in both the CF-KO and IL-10-KO mice seem similar, because both showed more profound consumption of I- κ B α and/or decreases in its reaccumulation, whereas I- κ B α reached supraphysiologic levels in both groups of WT mice. It is possible that studies earlier than 2 hours would show more profound differences that we may have missed because we focused on the prolonged response at later times. The prolonged decreases in I- κ B α correspond to the prolonged and excessive activation of NF- κ B in both types of knockout mice. Thus, once CF-KO and IL-10-KO mice degrade their lung I- κ B α in response to an acute transient stimulus, their ability to regenerate and/or reaccumulate this important inhibitory molecule is impaired. This allows exaggerated and prolonged NF- κ B activation and prolonged proinflammatory cytokine synthesis. Although epithelial cells are not likely to be the sole sources of proinflammatory cytokines, because we used tissue remaining after extensive BAL and perfusion, we believe that the airway epithelium is an important site of this NF- κ B activation. However, we cannot rule out some contribution by transiting inflammatory cells. Expression of NF- κ B-dependent inflammatory mediators in epithelial cells has been shown in 2 recent studies by using transgenic mice specifically targeted to have impaired NF- κ B activation in lung epithelial cells rather than macrophages, and lung epithelial cells are important sites of cytokine synthesis after inhaled LPS challenge.^{26,27} Our results are also consistent with reports of exaggerated activation of NF- κ B in response to *P aeruginosa* and TNF- α in primary human CF bronchial epithelial cells and CF cell lines.^{28,29}

Our findings suggest that inhibition of I- κ kinase and/or induction of I- κ B α synthesis after it has been consumed after acute challenge might be important mechanisms by which IL-10 exerts anti-inflammatory effects in the normal lung. These conclusions are supported by other *in vivo* and *in vitro* studies of IL-10.^{15,24,25,30} Treatment of CF-KO mice with IL-10 attenuated the excessive in-

flammatory response to LPS, decreasing the PMNs, reducing the TNF- α production, and inhibiting NF- κ B activation. This was associated with an increased content of I- κ B α protein in lungs. The results do not allow us to distinguish between decreased degradation of I- κ B versus increased replacement synthesis as actions of IL-10. However, our results corroborate studies in transgenic mice that overexpress IL-10 in the lung³⁰ or in which exogenous IL-10 was added to primary bronchial epithelial cells from patients with CF *in vitro*.³¹ In both cases, IL-10 diminished inflammatory cytokine production. However, unlike studies in which IL-10 was chronically overexpressed in the lung *in vivo*,³⁰ transient systemic treatment with IL-10 caused no deleterious effects.

In summary, the response of both IL-10-KO and CF-KO mice to acute LPS challenge is excessive and resembles that found in BAL of chronically infected patients with CF.^{3,7,19} Thus, our studies of the signaling pathways, which showed more profound consumption and/or decreased replacement of I- κ B α and increased and prolonged NF- κ B activation, may also be applicable to patients with CF. Future experiments should help elucidate the mechanisms by which CFTR defects lead to IL-10 deficiency in the lung and the mechanisms by which this cytokine modulates I- κ B α and NF- κ B activation *in vivo*.

We thank Dr Marck Shluchter for help with statistical analysis, Jay Hilliard and Alma Gente Wilson, and Anna van Heeckeren and other members of the Animal Core of the Case Western Reserve University Cystic Fibrosis Center for providing us with mice.

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