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Targeted Immunomodulation of the NF-κB Pathway in Airway Epithelium Impacts Host Defense against Pseudomonas aeruginosa

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We investigated the impact of inflammatory signaling in airway epithelial cells on host defense against Pseudomonas aeruginosa, a major cause of nosocomial pneumonia. In mice, airway instillation of P. aeruginosa resulted in NF-κB activation in the lungs that was primarily localized to the bronchial epithelium at 4 h, but was present in a variety of cell types by 24 h. We modulated NF-κB activity in airway epithelium by intratracheal delivery of adenoviral vectors expressing RelA (AdRelA) or a dominant inhibitor of NF-κB before P. aeruginosa infection. Bacterial clearance was enhanced by up-regulation of NF-κB activity following AdRelA administration and was impaired by treatment with a dominant inhibitor of NF-κB. The TNF-α concentration in lung lavage was increased by AdRelA treatment and beneficial effects of NF-κB up-regulation were abrogated in TNF-α-deficient mice. In contrast, NF-κB inhibition reduced MIP-2 expression and neutrophil influx following P. aeruginosa infection. Therefore, inflammatory signaling through the NF-κB pathway in airway epithelial cells critically regulates the innate immune response to P. aeruginosa. The Journal of Immunology, 2006, 176: 4923–4930.

Pseudomonas aeruginosa is an opportunistic pathogen that causes disease in patients with impaired host defenses and is often a cause of life-threatening nosocomial infection in critically ill and immunocompromised patients (1–4). It is also the leading cause of mortality and morbidity in patients with cystic fibrosis (5–7). Due to the ubiquitous nature of P. aeruginosa and its ability to develop resistance to antibiotics, it continues to be problematic from a treatment perspective. Although the pathobiology of P. aeruginosa pneumonia involves complex interactions between a variety of bacterial and host factors (8, 9), the pivotal role of airway epithelial cells, including epithelial cells, in the innate immune response to P. aeruginosa is now beginning to be understood.

Bronchial epithelial cells are capable of sensing infecting organisms and mobilizing an innate immune response through the secretion of effector molecules (10, 11). Infection with P. aeruginosa results in activation of signaling cascades in epithelial cells that lead to expression of cytokines, chemokines, and defensins (12), and may induce apoptosis (13, 14). In vitro studies have shown that interaction of P. aeruginosa with specific receptors on epithelial cells is a potent stimulus for nuclear translocation of the transcription factor, NF-κB (15, 16). However, the role of airway epithelium in vivo has not been well characterized and the impact of specific signal transduction pathways, such as NF-κB, on generation of an effective innate immune response is unknown. NF-κB comprises a family of transcription factors that regulates expression of genes involved in inflammatory/immune responses (17). Usually, the term NF-κB is collectively used for homo- and heterodimeric complexes formed by Rel/NF-κB proteins. Signal-induced NF-κB activation is mainly accomplished by phosphorylation of IkB proteins at two specific serine residues, followed by polyubiquitination and degradation by the 26S proteasome. Liberated NF-κB then translocates to the nucleus, where it modulates gene expression by binding to κB motifs in promoters of target genes (18–20).

Because NF-κB is a key regulator of many important host defense genes and P. aeruginosa is known to activate NF-κB in epithelial cells, we hypothesized that modulation of NF-κB in airway epithelium would impact bacterial clearance in the lungs by altering the innate immune response following infection. Although bacterial components are known to generate a host response by activation of NF-κB via TLR in macrophages and other inflammatory cells, it is becoming increasingly apparent that NF-κB signaling in structural cells, including epithelial cells, is necessary to mount a robust inflammatory response in the lungs (21, 22). Previously, we have shown that activation of NF-κB in airway epithelium is sufficient to induce neutrophilic lung inflammation (23). The present studies were designed to determine whether selectively intervening in the NF-κB pathway in airway lung epithelium in vivo alters the pathogenesis of P. aeruginosa pneumonia.

Materials and Methods

Animal model

Wild-type (WT) C57B6/Dba mice or transgenic NF-κB reporter mice weighing 20–30 g were used. Transgenic mice expressing Photinus luciferase

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Abbreviations used in this paper: WT, wild type; IT, intratracheal; Ad, adenovirus; BAL, bronchoalveolar lavage; RLU, relative light unit.
cDNA under control of the proximal 5' HIV-LTR mite on a C57Bl/6 DBA background (HLL) were used (24). Another transgenic mouse line (NGL) used in some studies contains a construct in which four copies of the HIV-LTR NF-κB enhancer element were placed upstream of the herpes virus thymidine kinase minimal promoter to drive expression of enhanced GFP-luciferase fusion protein (25). Additional studies were done using TNF-α-deficient mice (C57Bl/6 background) obtained from Dr. M. Martin (Sloan-Kettering Cancer Center, New York, NY), and WT (C57Bl/6) controls (26, 27).

Mice were treated with intratracheal (IT) administration of adenoviral vectors after sedation with ketamine/xylazine. Mouse tracheas were directly exposed by surgical resection, pierced with a 26-gauge needle, and injected with 100 μl of the adeno virus (Ad) preparation diluted in sterile PBS. The neck wound was closed with sterile sutures under aseptic conditions.

At the conclusion of experiments, mice were euthanized and lungs were removed; one lung was ground in 1 ml of ml of reporter lysis buffer (Promega) and stored at −20°C for luciferase assays, bacterial colony counts, and the other lung was frozen at −70°C. In some experiments tracheas were cannulated and lungs were lavaged in situ with sterile pyrogen-free physiological saline that was instilled in three 1-ml aliquots and gently withdrawn with a 1-ml syringe. The studies were approved by the Vanderbilt Institutional Animal Care and Utilization Committee. The mite was mounted and photons were counted over a standard area corresponding to the region of the chest overlying the mid lung zone. Photon counts were obtained before and following treatment with adenoviruses so that each mouse could be used as its own control.

Measurement of luciferase activity
Luciferase activity was measured in cells or postmortem tissue samples by adding 100 μl of freshly reconstituted luciferase assay buffer to 20 μl of cell lysate or homogenated lung tissue that was ground in 1 ml of isotonic saline (28). Luciferase activity was expressed as relative light units normalized for protein content, which was measured by Bradford assay.

Total and differential cell counts in bronchoalveolar lavage (BAL)
BAL fluid was centrifuged at 400 x g for 10 min to separate cells from supernatant. Supernatant was saved separately and frozen at −70°C. The cell pellet was suspended in serum-free RPMI 1640 culture medium and total cell counts were determined on a grid hemocytometer. Differential cell counts were determined by staining cytospin slides with a modified Wright stain (Diff-Quick; Baxter) and counting 400–600 cells in complete cross-sections.

Chemokine and cytokine ELISAs
MIP-2, IL-β, IL-12 (p70 and p40), and TNF-α levels in BAL were measured using specific ELISA kits according to the manufacturer’s instructions (R&D Systems).

Extraction of nuclear proteins and Western blot for RelA
Nuclear proteins were extracted from whole-lung tissue. A total of 50–100 mg of tissue was mechanically homogenized in liquid nitrogen, to which 4 ml of buffer A (150 mM NaCl, 10 mM HEPES (pH 7.9), 0.6% (v/v) Nonidet P-40, 0.2 M EDTA, 0.1 M PMSF) was added. The homogenate was transferred to a 15-ml Falcon tube and centrifuged at 850 x g in a tabletop centrifuge for 10 min to separate cells from nuclear extract. The supernatant was then transferred to a 50-ml Falcon tube and incubated on ice for 5 min before being centrifuged for 10 min at 3500 x g. Supernatant was collected as a cytoplasmic extract. The pellet was resuspended in 300 μl of buffer B (sterile water, 25% (v/v) glycerol, 20 mM HEPES (pH 7.9), 5 min NaCl, 1 M MgCl₂, 0.2 min EDTA, 0.1 M phenylsulfonyl fluoride, 1 mg of minidithiothreitol, 10 mg of benzenamide/ml, 1 mg of pepstatin/ml, 1 mg of leupeptin/ml, 1 mg of aprotinin/ml) and incubated on ice for 30 min. Following centrifugation at 14,000 rpm in an Eppendorf microcentrifuge for 2 min, the supernatant was collected as the nuclear extract and was frozen at −70°C. Protein concentrations in nuclear extracts were determined by using the Bradford assay. For Western blot, 25 μg of nuclear protein was separated on a 10% acrylamide gel, transblotted and immunodetected. Abs to RelA were obtained from Santa Cruz Biotechnology.

Statistical analysis
Our statistical analyses were performed with GraphPad InStat software, version 3.01 for Windows NT (GraphPad) using an unpaired t test and unpaired ANOVA. Values of p < 0.05 were considered significant.

Results
To evaluate induction of NF-κB-dependent transcriptional activity in lung epithelial cells by P. aeruginosa (strain PA103), we treated A549 cells with adenoviral vectors expressing luciferase under the control of an NF-κB-dependent promoter (NF-κBluc) were a gift from Dr. L. Prince (University of Alabama, Birmingham, AL) (28, 29). Adenoviral vectors expressing luciferase were a gift from Dr. A. Powers (Vanderbilt University, Nashville, TN). Adenoviral vectors were propagated, purified, and stored at −70°C. Luciferin (1 mg/mouse in 200 μl of isotonic saline) was administered i.p. injection and mice were imaged with an intensified charge coupling device camera (model no. C2400-32; Hamamatsu). For the duration of photon counting, mice were placed inside a light tight box. Light emission from the mouse was detected as photon counts using the intensified charge coupling device camera and customized image processing hardware and software (Hamamatsu). A digital false-color photon emission image of the lungs of mice following IT infection with P. aeruginosa. To assess NF-κB-dependent transcriptional activity in individual cells in vivo, transgenic reporter mice that express GFP-luciferase fusion protein under the control of an NF-κB-dependent promoter were infected with PA103 (10⁶ CFU) and lungs were harvested at

Histology and GFP immunohistochemistry
To collect lung tissue, mice were perfused with saline and lungs were inflated with 1 ml of 10% neutral-buffered formalin. After paraﬁn embedding, 5-μm sections were cut and placed on charged slides. Following parafﬁn removal, sections were rehydrated and placed in heated Target Retrieval Solution, High pH (DakoCytomation) for 20 min. Tissues were incubated with anti-GFP Ab (BD Clontech) for 2 h at room temperature. Sections without primary Ab served as negative controls. The Vectastain ABC Elite (Vector Laboratories) System and DAB’ (DakoCytomation) were used to produce localized, visible staining. Slides then were lightly counterstained with Mayer’s hematoxylin, dehydrated, and coverslipped.

Bioluminescence imaging
Mice were anesthetized and shaved over the chest and abdomen before imaging. Luciferin (1 mg/mouse in 200 μl of isotonic saline) was administered by i.p. injection and mice were imaged with an intensified charge couple device camera (model no. C2400-32; Hamamatsu). For the duration of photon counting, mice were placed inside a light tight box. Light emission from the mouse was detected as photon counts using the intensified charge coupling device camera and customized image processing hardware and software (Hamamatsu). A digital false-color photon emission image of

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By immunohistochemical detection, GFP was expressed predominantly in bronchial epithelial cells at 4 h; however, by 24 h, NF-κB-dependent GFP expression was identified in epithelium and a variety of other cell types, particularly macrophages and infiltrating neutrophils (Fig. 1a). These data indicate that initial NF-κB activation occurs in epithelial cells following airway challenge with P. aeruginosa and suggests that the NF-κB pathway in epithelial cells is positioned to play a central role in coordinating the host response to P. aeruginosa.

After identifying prominent NF-κB activation in airway epithelial cells following P. aeruginosa infection, we used adenoviral vectors to target the NF-κB pathway in these cells. First, we used a construct that contains an NF-κB reporter to determine whether P. aeruginosa induces NF-κB activation in cells that express transgenes delivered by adenoviral vectors. AdNF-κBΔluc (10⁹ PFU) was administered by IT injection into WT mice 48 h before challenge with P. aeruginosa. Following i.p. injection of 1 mg of luciferin, in vivo luciferase activity was detected by bioluminescence imaging. No increase in chest photon emission over baseline was detected at 48 h after AdNF-κBΔluc injection (not shown). P. aeruginosa or control (PBS) was then administered intratracheally and bioluminescence was re-evaluated 24 h later. A marked increase in photon emission from the lungs was detected 24 h after P. aeruginosa infection compared with the same mice before P. aeruginosa infection or controls treated with IT administration of PBS (Fig. 1b). These findings were supported by postmortem luciferase activity measurements in lung homogenates, which revealed luciferase activity of 42 ± 7 relative light units (RLU)/μg of protein in mice treated with PBS compared with 285 ± 38 RLU/μg protein in mice treated with P. aeruginosa, p < 0.001 (Fig. 1c). Together, these experiments indicate that intratracheally delivered adenoviral vectors target a population of airway epithelial cells that activate NF-κB as a consequence of P. aeruginosa infection.

To modulate NF-κB activation in airway epithelial cells, we used adenoviral vectors that express RelA, which is the transactivating component of the NF-κB heterodimer, or adenoviral vectors expressing a dominant inhibitor of NF-κB (AdIκBdn) (23). Transgenic NF-κB reporter mice that express luciferase under the control of an NF-κB promoter (HLL) (24, 30) were treated by IT injection of PBS or adenoviral vectors (10⁹ PFU) expressing RelA, IκBdn, or a control (β-gal). In previous studies using this method of IT adenoviral injection, we showed that transgene expression occurs selectively in airway epithelium and not other cell types, including alveolar macrophages (23). To rule out a direct effect of adenoviral vectors on alveolar macrophages in the current studies, we evaluated NF-κB activation on alveolar macrophages (23). To modulate NF-κB activation in alveolar macrophages in the current studies, we used adenoviral vectors on alveolar macrophages in the current studies, including alveolar macrophages (23). To rule out a direct effect of adenoviral vectors on alveolar macrophages in the current studies, we evaluated NF-κB activation in alveolar macrophages (23). To rule out a direct effect of adenoviral vectors on alveolar macrophages in the current studies, we evaluated NF-κB activation in alveolar macrophages (23). To rule out a direct effect of adenoviral vectors on alveolar macrophages in the current studies, we evaluated NF-κB activation in alveolar macrophages (23). To rule out a direct effect of adenoviral vectors on alveolar macrophages in the current studies, we evaluated NF-κB activation in alveolar macrophages (23). To rule out a direct effect of adenoviral vectors on alveolar macrophages in the current studies, we evaluated NF-κB activation in alveolar macrophages (23). To rule out a direct effect of adenoviral vectors on alveolar macrophages in the current studies, we evaluated NF-κB activation in alveolar macrophages (23).

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and b). In addition, mice treated with AdIβBdn exhibited significantly less photon emission from the lungs than did mice treated with PBS or Adβ-gal at 24 h after *P. aeruginosa* infection. These data indicate: 1) total NF-κB activity in the lungs is up-regulated after treatment with AdRelA, and 2) *P. aeruginosa* induced NF-κB activity is significantly suppressed by administering viruses that express the dominant inhibitor of NF-κB.

To corroborate bioluminescent imaging for evaluation of NF-κB activity in these mice, luciferase activity was measured in lung homogenates and nuclear translocation of RelA was measured in...
lung nuclear protein extracts. At the time of harvest (24 h after *P. aeruginosa* infection), lung luciferase activity was 520 ± 75 RLU/μg protein for the AdRelA-treated group, 260 ± 51 RLU/μg protein for the Adβ-gal group, and 112 ± 35 RLU/μg protein for the AdIκBdn group (*p < 0.05 for both AdRelA and AdIκBdn groups compared with Adβ-gal). Although NF-κB-dependent lung luciferase activity was significantly higher in mice treated with AdRelA compared with mice that were treated with control adenoviral vectors (Adβ-gal) or AdIκBdn, mice treated with AdIκBdn contained less luciferase activity in the lungs than mice treated with Adβ-gal. By Western blot analysis for nuclear RelA, lungs from AdRelA-treated mice showed greater amounts of nuclear RelA than the other groups, and lungs from AdIκBdn-treated mice showed reduced nuclear RelA (Fig. 2, c and d). Together, these experiments indicate that IT administration of adenoviral vectors targeting the NF-κB pathway can substantially modulate *P. aeruginosa*-induced NF-κB activation in the lungs.

To determine whether modulation of epithelial cell NF-κB alters bacterial clearance after *P. aeruginosa* infection, we measured bacterial colony counts from the right middle lobe at 24 and 48 h after bacterial inoculation (Fig. 3). Although mice treated with Adβ-gal, AdRelA, AdIκBdn, or PBS received an equal initial dose of bacteria, AdRelA-treated mice showed significantly lower bacterial colony counts at 24 and 48 h compared with the other groups. At 48 h, mice treated with AdIκBdn had increased bacterial colony counts compared with controls treated with Adβ-gal or PBS before *P. aeruginosa*. No differences in colony counts were observed at 24 or 48 h between Adβ-gal- and PBS-treated mice. Because no differences in bacterial clearance (or measurements of NF-κB activation) were observed between mice treated with PBS or control adenoviral vectors (Adβ-gal) before *P. aeruginosa* infection, Adβ-gal-treated mice were used as a single control group for subsequent experiments. These data show that accentuation of NF-κB activity in airway epithelial cells enhances bacterial clearance whereas NF-κB inhibition impairs host defense in this model of *P. aeruginosa* infection.

We performed additional studies to evaluate mechanisms by which NF-κB modulation could impact clearance of *P. aeruginosa* from the lungs. BAL was performed at the time of harvest (24 h after infection) (Fig. 4a). At this time point, neutrophils account for the majority of BAL cells in all three treatment groups (Adβ-gal, AdRelA, or AdIκBdn). Although there was a trend toward increased total cells and neutrophils from BAL in mice that were treated with AdRelA compared with Adβ-gal before *P. aeruginosa* infection, mice treated with AdIκBdn showed significantly lower total cells and neutrophils in BAL compared with the other groups. We also measured concentrations of NF-κB-dependent mediators in BAL, including TNF-α, IL-1β, MIP-2, and IL-12. IL-1β and IL-12 levels were not different among the three groups. Interestingly, MIP-2 levels paralleled neutrophil counts with no significant differences between MIP-2 levels from mice treated with AdRelA and Adβ-gal, but decreased levels of MIP-2 in BAL from mice treated with AdIκBdn (Fig. 4b). For TNF-α, mice treated with AdRelA before *P. aeruginosa* infection developed significantly higher TNF-α levels in BAL compared with the other groups of mice (Fig. 4c).

**FIGURE 3.** Modulation of NF-κB activity in airway epithelial cells alters the pathogenesis of *P. aeruginosa* pneumonia. Bacterial colony counts from the right middle lobe 24 and 48 h after IT administration of *P. aeruginosa* (PA103) into mice that were pretreated with Adβ-gal, AdRelA, AdIκBdn, or PBS. Initial colony counts were obtained by harvesting a group of mice 30 min after infection (*, *p < 0.05 compared with Adβ-gal and PBS controls, n = 6/group).
Discussion

In these studies, we found that infection with *P. aeruginosa* induced NF-κB activity in epithelial cells in vivo. Early epithelial NF-κB activity was followed by recruitment of inflammatory cells and further NF-κB activation in a variety of cell types. By modulating epithelial NF-κB activity in response to bacterial challenge, we identified NF-κB signaling in these cells as a major determinant of the outcome of *P. aeruginosa* pneumonia. Up-regulation of NF-κB activity improved bacterial clearance, while blockade of NF-κB activation impaired lung host defense. The beneficial effects of accentuated NF-κB activation appeared to be related to increased production of TNF-α, because BAL levels of TNF-α were increased in mice treated with AdRelA and beneficial effects of AdRelA treatment were abrogated in TNF-α-deficient mice. In contrast, impaired clearance of bacteria following epithelial NF-κB blockade may be related to decreased neutrophil recruitment because lower MIP-2 levels and reduced neutrophil counts were found in BAL following treatment with AdIκBdn.

Airway epithelium is involved in innate immunity in the lungs in multiple ways: establishing a physical barrier to microbial invasion, providing a structural basis for mucociliary clearance, recognizing microbial products by pattern recognition receptors, secreting antimicrobial substances including defensins, and producing a variety of proinflammatory mediators (11). Recent studies have highlighted specific epithelial processes, such as internalization of bacteria and apoptosis mediated through the CD95 pathway, in effective host defense against *P. aeruginosa* (13, 14).

Our studies, however, focus on the role of the airway epithelium in initiating and regulating inflammatory signaling in the lungs, functions whose importance is being increasingly recognized (21, 22). The expression of TLRs 1–10 by airway epithelial cells (31, 32) indicates that these cells are likely participants in inflammatory signaling in infected lungs. *P. aeruginosa* products, including pili and flagella, can bind to TLRs on bronchial epithelial cells through an interaction with asialyted glycolipids such as asialoGM1 (33, 34). Interaction with these receptor complexes, present in lipid rafts, leads to downstream activation of NF-κB and production of inflammatory cytokines (33, 35). Our studies indicate that signaling through the NF-κB pathway in epithelial cells plays an important host protective role against *P. aeruginosa* infection in intact animals.

Several recent investigations have implicated the NF-κB pathway in host defense against lung pathogens, including *P. aeruginosa* (36–41). Skerrett et al. (36) reported that MyD88, an adaptor protein that facilitates TLR-dependent NF-κB activation, was essential for clearance of *P. aeruginosa* from the lungs. Using MyD88-deficient mice, this group showed that MyD88-dependent signaling was integral to cytokine production and neutrophil recruitment in response to respiratory tract infections with *P. aeruginosa* and *Staphylococcus aureus*; however, MyD88 was required for clearance of *P. aeruginosa* but not *S. aureus* (36). Using a similar study design, Power et al. (37) confirmed that MyD88-deficient mice have impaired bacterial clearance of *P. aeruginosa* from the lungs compared with the WT controls. This defect in host defense was found to be related to globally deficient activation of NF-κB in the lungs and reduced production of MIP-2, TNF-α and IL-1β. In humans, mutations in genes encoding IRAK-4, an upstream kinase that leads to activation of NF-κB and the NF-κB essential modulator, have been linked with impaired NF-κB activation and recurrent infections with Gram-positive and -negative bacteria (including *P. aeruginosa*) (38–41). Together, these studies imply that NF-κB signaling is important for generating a protective innate immune response to bacterial invasion. Our studies further define this connection between NF-κB and host defense by providing direct evidence of a host protective function of NF-κB activation in a defined cell population.

Although the NF-κB pathway impacts a variety of cellular processes and influences transcription of many genes, our data suggest that the beneficial effects of up-regulating NF-κB activity in airway epithelium are related to increased TNF-α production. In our model, it is unclear whether augmented TNF-α production following AdRelA treatment results from direct up-regulation of NF-κB-dependent transcription in epithelial cells or from downstream processes.
stimulation of alveolar macrophages or other immune cells by mediators released from the epithelium. In A549 epithelial cells, neither infection with AdRelA nor addition of P. aeruginosa were found to induce TNF-α expression (data not shown). Although the source of TNF-α is unclear, this mediator has been previously implicated in host defense against P. aeruginosa. The administration of adenoviral vectors expressing TNF-α to mice before IT challenge with P. aeruginosa enhances bacterial clearance (42), whereas administration of TNF-α-neutralizing Ab is associated with impaired bacterial clearance (43). Interestingly, TNF-α knockout mice exhibit a major defect in P. aeruginosa clearance (44) but mice deficient in TNFRs are not more susceptible than controls to P. aeruginosa infection (45, 46). In a recent study, patients with cystic fibrosis who had additional polymorphisms in the TNF-α gene had an increased susceptibility to P. aeruginosa with severe lung disease compared with the patients who had normal expression of TNF-α (47). Together, these studies implicate TNF-α as an important effector molecule in host defense against P. aeruginosa.

In mice treated with the NF-κB inhibitor (AdIκBdn), TNF-α levels were similar to controls following P. aeruginosa infection; however, decreased expression of the neutrophil chemotactic chemokine MIP-2 and impaired neutrophil recruitment were observed. Recruitment of neutrophils by CXC chemokines is a major component of the protective innate immune response to P. aeruginosa (48, 49). Thus, impaired host defense in AdIκBdn-treated mice is likely related to deficient neutrophil recruitment. Although NF-κB-dependent signaling in the airway epithelium is required for maximal MIP-2 production following P. aeruginosa infection, further up-regulation of NF-κB by AdRelA treatment did not result in additional chemokine expression and neutrophil recruitment.

In these studies, we have defined the NF-κB pathway in airway epithelial cells as a specific target for immunomodulatory therapy in P. aeruginosa pneumonia. However, further studies are needed to fully identify the mechanisms by which NF-κB contributes to host defense and bacterial clearance. By carefully defining targets for intervention, immunomodulatory therapies may prove beneficial as adjuvant therapies to treat patients with severe or recurrent P. aeruginosa infections.

Disclosures
The authors have no financial conflict of interest.

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3. Afessa, B., and B. Green. 2000. Bacterial pneumonia in hospitalized patients with severe lung disease compared with the patients who had normal expression of TNF-α (47). Together, these studies implicate TNF-α as an important effector molecule in host defense against P. aeruginosa.

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