Induction of Type I Interferon Signaling by *Pseudomonas aeruginosa* Is Diminished in Cystic Fibrosis Epithelial Cells

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The clinical manifestations of infection in cystic fibrosis (CF) are restricted to the lung, and involve a limited number of pathogens, suggesting a specific defect in mucosal immunity. We postulated that cystic fibrosis transmembrane conductance regulator (CFTR) mutations could affect the activation of type I interferon signaling in airway epithelial cells, which function in immune surveillance and initiate the recruitment and activation of immune cells. In response to infection with *Pseudomonas aeruginosa*, *Ifnb* was induced more than 100-fold in the murine lung, and the phosphorylation of STAT1 was similarly induced by the expected TLR4/TRIF/MD2/TBK1 cascade. The stimulation by *P. aeruginosa* of CF (IB3) cells and control (C-38) human cell lines similarly resulted in the induction of IFN- β , but to a significantly lower extent in CF airway cells. The potential consequences of diminished type I IFN signaling were demonstrated in a murine model of *P. aeruginosa* pneumonia, pretreatment with polyinosinic:polycytidylic acid significantly enhanced bacterial clearance and correlated with increased numbers of mature CD11c⁺/CD86⁺ dendritic cells (DCs) in the lung. Using culture supernatants from CF or control cell lines stimulated with *P. aeruginosa*, we similarly demonstrated the diminished activation of human monocyte-derived DCs by incubation with CF compared with normal epithelial cell culture supernatants, which was dependent on IFN- β . These observations suggest that dysfunction of the CFTR in airway epithelial cells may contribute to impaired immune surveillance in the CF airway and resultant colonization by *P. aeruginosa*.

Keywords: Type I interferon; cystic fibrosis; *Pseudomonas aeruginosa*; TLR4

A major component of cystic fibrosis (CF) pathophysiology involves defective mucosal immunity. Bacteria are inhaled and persist in the airways, despite the appearance of normal immune function. Current hypotheses suggest that organisms such as *Pseudomonas aeruginosa* can adapt and proliferate in the relatively dehydrated CF airway surface fluid more readily than in the normal lung. These organisms or their shed components stimulate the expression of epithelial chemokines (1) and activate a Th17 response, marked by increased concentrations of IL-17 and IL-23 in bronchoalveolar lavage (1). Signaling from these epithelial cells and T cells is critical in up-regulating granulocytopoiesis (2).

CLINICAL RELEVANCE

We demonstrate that *Pseudomonas aeruginosa* is able to activate type I IFN signaling in the airway. This pathway is important for clearance of the organism, and this signaling is abrogated in epithelial cells with cystic fibrosis transmembrane conductance regulator mutations. This work provides a new mechanism to explain the poor response of patients with cystic fibrosis to bacterial infections, and in particular to *P. aeruginosa*.

It remains unclear why initial innate immune defenses are not effective in clearing inhaled bacteria early in the disease process, before substantial mucus plugging and airway damage occur. Clinical data and *in vitro* studies demonstrated a hyperinflammatory milieu in CF airways and an endogenous up-regulation of NF- κ B in airway cells (3–9), even before clinical evidence of infection appears (10). Thus, it seems paradoxical that bacteria inhaled into CF lungs already populated by polymorphonuclear leukocytes (PMNs) are not immediately ingested and cleared. Whether mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) affect phagocyte function has been debated (11, 12), and no clinical evidence is available that immune function in CF is abnormal outside of the lung.

The type I IFN cascade is an important component of the innate immune system that protects mucosal surfaces (13, 14). The role of type I IFNs (α and β) and their common receptor in antiviral innate immunity is well-established, and evidence is increasing that the components of extracellular bacteria also stimulate the production of type I IFN in airway epithelial and immune cells. *Staphylococcus aureus* protein A potentially activates the type I IFN cascade (1), as does DNA from Group B streptococcal DNA (15) and *S. pneumoniae* (16). The induction of type I IFN responses in the respiratory tract is initiated by intracellular receptors of several different types within mucosal epithelial cells (17). These include Toll-like receptors (TLRs) linked to the TRIF/TRAM adaptors in endosomes, nucleotide binding and oligomerisation domain proteins that respond to peptidoglycan fragments, bacterial DNA, and other ligands. The TRIF adapter was shown to be involved in the clearance of *P. aeruginosa* (18).

Type I IFN signaling involves the expression of more than 300 genes that exert both proinflammatory and anti-inflammatory effects (19, 20). A key role of IFN- β and other type I IFN effectors involves activating dendritic cells (DCs) in the airways, which then direct the recruitment and activation of appropriate responses by T-cells (21). Substantial data indicate the importance of Th1 and especially of Th17 signaling in the effective clearance of extracellular bacterial pathogens from the airways (22). The impaired activity of DCs would affect responses by T-cells to inhaled pathogens. Other functions of these DCs include

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Figure 1. *Pseudomonas aeruginosa* activates type I IFN signaling in the lung and *in vitro*. C57BL/6J mice were infected with 10⁷ colony-forming units (CFUs) of *P. aeruginosa* (P.a.) strain PAK or 50 μg of LPS for 16 hours before being killed. RNA from infected lungs was analyzed by quantitative RT-PCR for (A) *Ifnb*, (B) *Mx1*, and (C) *Il6*. Each point represents an individual mouse, and lines indicate median values. The combined results from two independent experiments are shown ($n = 6$). (D) Proteins from lung homogenates were analyzed by immunoblot for phosphorylation of the transcription factors STAT 1, 2, and 3. β-actin was used as a loading control. Each lane represents an individual mouse. A representative blot is depicted. (E) Cultured murine nasal epithelial cells after 1 and 4 hours of exposure to *P. aeruginosa* (P.a.) ($n = 3$). (F) Murine dendritic cells (DCs) after 2 hours of exposure to *P. aeruginosa* (P.a.) or purified *E. coli* LPS ($n = 2$). Representative findings from two independent experiments are shown. * $P < 0.05$, compared with PBS or unstimulated samples. UN, unstimulated control.

regulating the influx of PMNs and rates of apoptosis. Exposure to LPS, as would occur in CF airways, induces the maturation and apoptosis of DCs, events that are critically involved in the development of tolerance versus immunogenicity (23, 24). Airway epithelial cells are an important source of type I IFN effectors (25), and their expression is affected by CFTR mutations. The induction of both regulated upon activation, normal T-cell expressed and secreted protein (RANTES) (26, 27) and the phosphorylation of STAT1 (28) are decreased in cells with CFTR mutations. We postulated that specific defects in epithelial type I IFN signaling because of a CFTR mutation may result in the impaired activation of surveillance by DCs in the airways. In this report, we demonstrate that a defect exists in the activation of type I IFN signaling in CF epithelial cells in response to LPS or to *P. aeruginosa*. This defect contributes to the diminished activation of pulmonary DCs, cells critical for immune surveillance and for orchestrating appropriate T-cell signaling.

MATERIALS AND METHODS

Cell Culture

IB3 (ΔF508/ W1282X) or C38 (corrected) CF epithelial cell lines were grown between passages 22 and 60 in LHC-8 media, 10% FBS, penicillin, streptomycin, and L-glutamine. Murine nasal epithelial cells from septa were isolated from the indicated strains of adult mice and grown polarized, as described elsewhere (29). CFBE410⁻ (ΔF508/ΔF508) cells stably expressing wild-type (WT) or ΔF508 CFTR (30, 31) were cultured in minimum essential media with 10% FBS, penicillin, and streptomycin, and 2 μg/ml puromycin. Epithelial cells were stimulated with 5 × 10⁷ CFUs/ml of *P. aeruginosa* strain PAK, 50 μg/ml LPS, or 25 μg/ml of polyinosinic:polycytidylic acid (poly[I:C]) as controls.

DCs were generated from bone marrow isolated from the femurs and tibias of mice. Bone marrow from *Cftr*-null mice was kindly provided by Scott Randell (University of North Carolina). DCs were cultured for 7 days in RPMI-640 media supplemented with 10% FBS, streptomycin, penicillin, and 20 ng/ml of granulocyte macrophage colony-stimulating factor (GM-CSF) (Peprotech, Rocky Hill, NJ). Immortalized macrophage cell lines

Figure 2. *P. aeruginosa* initiates type I IFN signaling through the Toll-like receptor (TLR)-4 complex. (A) Bone marrow-derived DCs from mice lacking TLR4, TRIF, MD2, and CD14 were stimulated with *P. aeruginosa* (P.a.) or LPS for 2 hours before analyzing RNA for concentrations of *Ifnb* quantitated by quantitative RT-PCR. (B) TBK1-null macrophage cell lines and control samples were stimulated with *P. aeruginosa* (P.a.) or LPS for 2 hours, and concentrations of *Ifnb* were quantified by quantitative RT-PCR. Graphs show means ($n = 3$) and SDs of representative data from two independent experiments. * $P < 0.05$, compared with respective wild-type (WT) stimulated cells. UN, unstimulated control.

were prepared as described previously (32), and were grown in Dulbecco's Modified Eagle's Medium with 10% FBS, penicillin, and streptomycin. Immune cells were stimulated with 2×10^7 CFUs/ml of *P. aeruginosa* PA01 (because of reduced cytotoxicity) and 100 ng/ml of LPS.

Human DCs were isolated from 40 ml of heparinized blood from healthy anonymous volunteers (blood collection approved by Columbia University IRB-AAAC5450) (33). DCs were cultured for 8 days in RPMI-1640 medium supplemented with 10% FBS, penicillin, streptomycin, 800 U/ml GM-CSF, and 500 U/ml IL-4 (R&D Systems, Minneapolis, MN). DCs were stimulated with filtered, polymyxin B-treated supernatant from C38 and IB3 (unstimulated and *P. aeruginosa*-stimulated) monolayers. Antibody neutralization experiments used 20 μ g/ml of anti-IFN- β (Pbl InterferonSource, Piscataway, NJ) or isotype-matched IgG (Santa Cruz Biotechnology, Santa Cruz, CA). The FACS analysis of human DCs was performed as described later, using phycoerythrin-labeled anti-CD11c (MHCD11c04; Caltag Laboratories, Carlsbad, CA) and FITC-labeled anti-CD86 (MHCD8601; Caltag Laboratories).

ELISA Conditions

Epithelial cells were stimulated with *P. aeruginosa* or LPS for the indicated times, under the conditions already described. Supernatants were collected for 24 hours, and cytokines were quantitated with DuoSets (R&D Systems), according to the manufacturer's instructions.

Murine Studies

P. aeruginosa lung infections were performed using 6-week-old C57BL/6J mice. MD2 and CD14 null mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were anesthetized with 100 mg/kg ketamine and 5 mg/kg xylazine, and inoculated with $0.3\text{--}1 \times 10^7$ CFUs of organism or LPS (50 μ g per mouse) intranasally before being killed

16–24 hours after infection. Poly(I:C) experiments were performed with mice that had undergone 24 hours of pretreatment with poly (I:C) before bacterial infection. FACS analysis was performed on leukocytes from homogenized lung samples (please see the online supplement).

Statistical Analysis

The significance of data that followed a normal distribution was determined using a two-tailed Student *t* test, and the significance of data that did not follow normal distribution was determined using a nonparametric Mann-Whitney test. For experiments involving more than one comparison, we used one-way ANOVA followed by a Dunn multiple comparison test. Statistics were performed with Prism software (GraphPad, La Jolla, CA).

RESULTS

P. aeruginosa Activates Type I IFN-Associated Gene Products in the Lung

To establish whether *P. aeruginosa* activates type I IFN signaling in the lung, we infected C57BL/6J mice with $0.3\text{--}1 \times 10^7$ CFUs of strain PAK, and monitored whole-lung lysates 16 hours after inoculation for the induction of type I IFN gene products (Figure 1). A 70-fold induction of transcription occurred, compared with PBS control samples (Figure 1A, $P = 0.0022$), and a slightly lower induction in response to LPS (*Escherichia coli*), which was specifically chosen to function as a known positive control. Other type I IFN gene products were also induced with a greater than 100-fold induction of *Mx1* (Figure 1B), a large GTPase that interferes with viral gene transcription.

Figure 3. Induction of type I IFN signaling by *P. aeruginosa* is reduced in cystic fibrosis epithelial cells. (A) Confluent monolayers of IB3 ($\Delta F508$ /W1282X) or C38 (corrected) epithelial cell lines were infected with *P. aeruginosa* (*P.a.*; 10^7 CFUs; $n = 3$) or LPS ($n = 2$) for 2 or 4 hours, and concentrations of *Ifnb* were quantified by quantitative RT-PCR. (B) C38 and IB3 cell lines were stimulated with *P. aeruginosa* (*P.a.*) for the given times or with LPS (4 hours), and protein lysates were analyzed for the phosphorylation of STAT1. β -actin was used as a loading control. (C) Concentrations of CXCL10 and CXCL8 were quantitated by ELISA from C38 and IB3 supernatants after stimulation with *P. aeruginosa* and LPS for the given times. (D) C38 and IB3 cell lines were stimulated with *P. aeruginosa* (*P.a.*) or LPS for 2 hours, and levels of IFN- λ (*Il29*) induction quantified by quantitative RT-PCR. (E) Bone marrow-derived DCs from heterozygous and homozygous *Cfrt*^{tm1UNC} mice were stimulated with *P. aeruginosa* (*P.a.*) for 2 hours, and concentrations of IFN- β were quantitated by quantitative RT-PCR. Results from two independent experiments are depicted ($n = 5$). UN, unstimulated. Graphs and immunoblots are representative of two independent experiments. * $P < 0.05$ in IB3 compared with C38 cell lines.

Figure 4. Levels of type I IFN-regulated genes are reduced in cystic fibrosis cells. C38 and IB3 cell lines were stimulated with *P. aeruginosa* (P.a.) or LPS for 2 hours, and levels of cytokine transcription were determined by quantitative RT-PCR. * $P < 0.05$ ($n = 4$) in IB3 cells compared with C38 cells. UN, unstimulated.

An induction of *Il6* occurred (Figure 1C), but not of *PKR*, a gene product expressed in response to infection with influenza (13) (data not shown). The induction of *Ifnb* expression is typically followed by an autocrine pathway and the phosphorylation of STAT (34). In response to *P. aeruginosa* PAK or LPS, an increased phosphorylation of STAT1, STAT2, and STAT3 was demonstrated in lung lysates compared with a PBS-exposed control sample (Figure 1D).

Because epithelial cells as well as DCs and macrophages produce IFN- β (25), we screened cell types individually for their response to *P. aeruginosa*. By 1 hour of exposure, an almost 100-fold induction of *Ifnb* expression in murine airway epithelial cells occurred in primary culture in response to *P. aeruginosa* (Figure 1E). This persisted for at least 4 hours after exposure. Murine bone marrow-derived DCs from C57BL/6J mice were also readily stimulated by our *P. aeruginosa* strain or LPS to induce *Ifnb* (Figure 1F).

P. aeruginosa Activates via a TLR4/TRIF/MD2 Cascade

A number of intracellular receptors initiate type I IFN signaling in response to microbial pathogen-associated molecular patterns (PAMPs), which include bacterial DNA, peptidoglycan fragments, and LPS (35). LPS, which is abundantly shed in the airways by actively growing *P. aeruginosa*, seemed likely to be involved in the activation of the type I IFN cascade (36). LPS interacts with CD14 and stimulates via a TIR-domain-containing adapter-inducing interferon- β (TLR4/TRIF)/myeloid differentiation factor 2 (MD2) complex to induce the expression of IFN- β and associated genes (37). We tested the participation of individual components in this LPS signaling complex (18) in the response to *P. aeruginosa*, using bone marrow-derived DCs from the appropriate knockout mice (Figure 2A). The induction of *Ifnb* by *P. aeruginosa* was almost entirely dependent on TLR4/TRIF and MD2, and less dependent on CD14. The distal components of the cascade were found to signal via TANK binding kinase 1 (38) (Figure 2B). These *in vitro* studies suggest that intact *P. aeruginosa* and LPS activate type I IFN signaling by canonical TLR4/TRIF-MyD88-independent signaling.

Decreased Type I IFN Responses in Cells with CFTR Mutations

To determine if mutations in CFTR affect the induction of the type I IFN cascade, we compared the effects of *P. aeruginosa* or

LPS on the expression of *Ifnb* in matched C38 (corrected) and IB3 ($\Delta F508/W1282X$, containing the episomal vector) cell lines (39) (Figures 3A and 3B). After 2 hours of bacterial exposure, a 10-fold increase in *Ifnb* transcription occurred in the C38 but not IB3 cells ($P = 0.014$). Stimulation with LPS followed a similar pattern (Figures 3A and 3B). A similar result was obtained with CFBE41o⁻ stably expressing WT or $\Delta F508$ CFTR (Figure E1 in the online supplement). CFBE cells expressing WT CFTR produced higher levels of *Ifnb* transcription than the CFTR mutant in response to *P. aeruginosa* and LPS. The induction of STAT1 phosphorylation correlated with the mRNA results

Figure 5. TLR3-mediated type I IFN signaling is active in cystic fibrosis cells. (A) C38 and IB3 cell lines were stimulated with polyinosinic:polycytidylic acid (poly[I:C]) for 2 hours, and levels of *Ifnb* induction were quantified by quantitative RT-PCR. (B) C38 and IB3 cell lines were stimulated with poly(I:C) or LPS for 2 hours, and the phosphorylation (p) of interferon regulatory factor 3 (IRF3) was detected using immunoblotting. The phosphorylation of STAT1 in the presence poly(I:C) was also determined. β -actin was used as a loading control. * $P < 0.05$ ($n = 3$) for the IB3 compared with the C38 cell line. Graphs and immunoblots are representative of two independent experiments. UN, unstimulated.

(Figure 3B). Substantially less phosphorylated-STAT1 in IB3 cells was evident at 2 and 4 hours after stimulation (Figure 3B). Differences in protein expression were also apparent. As an indicator of type I IFN signaling, the IFN- β -regulated protein CXCL10 (IP-10) was significantly induced in C38 cells by *P. aeruginosa* (135-fold after 2 hours) or LPS (more than 200-fold) (Figure 3C). The expression of CXCL10 was significantly reduced ($P < 0.0001$) in the IB3 cell line (Figure 3C). This difference in protein expression between the C38 and IB3 lines was less apparent in NF- κ B-regulated CXCL8 (Figure 3C). The type III IFN- λ is highly expressed in epithelial cells, shares major signaling components with the type I IFN cascade, and plays an important role against viral pathogens (40, 41). The induction of IFN- λ (*Il29*) was also diminished in CF cells in response to either LPS or *P. aeruginosa* (Figure 3D). The defect in the activation of IFN- β appears limited to epithelial cells, because the induction of *Ifnb* in CF and control bone marrow-derived DCs was equivalent (Figure 3E).

Differences in induction of other type I IFN-dependent genes were also observed (Figure 4). Significantly decreased responses to both *P. aeruginosa* and LPS were evident for *RANTES* ($P = 0.0042$), IP-10 (*Cxcl10*) ($P = 0.0015$), *ICAM-1* ($P = 0.0034$), and *G-CSF* ($P = 0.0077$). A greater than 100-fold induction of *Cxcl1* expression by both the CF and corrected cell lines was evident. Neither PKR nor Mx1 was induced by LPS or *P. aeruginosa*, although endogenous levels of gene expression were decreased in the CF cells. *Il8* (*Cxcl8*) and *Il6*, genes predominantly activated by NF- κ B, were equivalently induced in both IB3 and C-38 cell lines.

The type I IFN cascade can be activated by other PAMPs such as double stranded RNA (dsRNA), which gain access to the appropriate intracellular receptors. To determine if the defect in CF cells was limited to the TLR4-associated ligands or else affects other TLRs that also signal through the TRIF adaptor, we tested the response of the IB3 and C38 cells to poly(I:C), an analogue of dsRNA. Poly(I:C) stimulates the production of IFN- β via TLR3/TRIF and the induction of interferon regulatory factor 3 (IRF3), to stimulate IFN- β transcription (42). The induction of *Ifnb* in response to poly(I:C) in C38 cells measured almost 1,000-fold and was only slightly less in IB3 cells (Figure 5A), which had 10-fold less expression at baseline, differences unlikely to be biologically meaningful. A modest difference in poly(I:C)-induced phosphorylation IRF-3 occurred, with enhanced responses in normal compared with CF cells, a defect more apparent when cells were stimulated with LPS (Figure 5B). Thus, the defect in type I IFN signaling appears to be predominantly associated with *P. aeruginosa* and LPS/TLR4 signaling, and not the virally induced cascade.

Effects of Type I IFN Signaling on *P. aeruginosa* Clearance from the Murine Lung

We postulated that the induction of type I interferon signaling would be beneficial in protection from *P. aeruginosa* infection. Studies of TRIF and IRF3 knockout mice showed a decreased ability to clear infections (18, 43). Accordingly, we used a gain-of-function model to determine if enhanced type I IFN signaling might facilitate the clearance of *P. aeruginosa*. C57BL/6J mice were pretreated with poly(I:C) (which activates the production

Figure 6. Stimulation of type I IFN improves clearance of *P. aeruginosa* in mice. C57BL/6J mice were treated intranasally with 25 μ g/mouse of poly(I:C) for 24 hours before infection with 10^7 CFUs of *P. aeruginosa* (*P.a.*). Sixteen hours later, (A) lungs and (B) spleens were enumerated for bacterial numbers. Lung homogenates were analyzed for the presence of (C) neutrophils, (D) macrophages, and (E) DCs, as well as the activation of macrophage and DC populations (F and G). * $P < 0.05$ ($n = 6$), compared with the PBS-only sample. Lines display median points. The data represent two independent experiments.

Figure 7. Activation of human peripheral blood DCs after treatment with epithelial cell supernatant requires type I IFN signaling. (A) Epithelial cells were stimulated with either PBS or *P. aeruginosa* for 2 hours before the supernatant (after filtration and treatment with polymyxin B) was applied to human DCs for 48 hours. * $P < 0.05$, compared with C38 cells stimulated with *P. aeruginosa*. (B) Supernatants from C38 cells were also pretreated with IgG or α IFN- β antibody before stimulation of DCs. * $P < 0.05$, compared with IgG-treated *P. aeruginosa* supernatant-stimulated cells. Data represent two independent experiments ($n \geq 3$; 10,000 cells per point). Lines display median points. UN, unstimulated; *P.a.*, *P. aeruginosa*.

of IFN- β via TLR3/TRIF), and 16 hours later we inoculated the mice with *P. aeruginosa*. The addition of poly(I:C) induced the production of *Ifnb* (Figure E2). The poly(I:C)-exposed mice exhibited significantly improved clearance of *P. aeruginosa* ($P = 0.0411$), with a 2-log decrease in pulmonary infection and significantly reduced bacteremia ($P = 0.0022$), compared with PBS-treated/*P. aeruginosa*-infected controls (Figures 6A and 6B). The recruitment of PMNs in *P. aeruginosa*-infected mice was not affected by pretreatment with poly(I:C) (Figure 6C), suggesting that the PMNs themselves are unlikely to be responsible for the increased bacterial clearance. The total macrophage and DC populations were not significantly increased by poly(I:C) (Figures 6D and 6E); but the concentration of CD86 (activation marker) in macrophages and DCs was significantly increased in poly(I:C)-exposed animals (Figures 6F and 6G).

Activation of Human DCs *In Vitro* by Epithelial Cell Products

These observations suggested that an LPS/TLR4-specific defect in the induction of IFN- β could affect the activation of DCs in the lung, which could ultimately affect bacterial clearance. To determine whether the differences in type I IFN gene products

induced by *P. aeruginosa* in IB3 and C38 cells affected the activation of DCs, we incubated DCs differentiated from normal human monocytes, with conditioned media harvested from IB3 and C38 cells after their exposure to *P. aeruginosa* (Figure 7A). After 48 hours of incubation, 235% more CD11c⁺/CD86⁺ cells (144,773 versus 61,665 per milliliter, $P = 0.0087$) had been activated in response to the *P. aeruginosa* C38-stimulated products than those exposed to IB3 cell products, consistent with the hypothesis that the epithelial activation of DCs may be deficient in CF. To determine whether type I IFNs, and specifically IFN- β , mediated this activation of DCs, the stimulated epithelial-cell media were treated with antibody against IFN- β or an isotype-matched control before incubation with DCs. Media treated with α IFN- β exhibited a significantly reduced ability to activate DCs, compared with the isotype control antibody (Figure 7B), indicating that the defect in CF epithelial cells reduces their ability to signal and activate DCs.

DISCUSSION

The induction of type I IFN signaling is an important component of the successful innate immune response to both bacterial and viral infections. The participation of this cascade in host defense against bacterial infection has been increasingly appreciated (44). Mucosal epithelial cells play a major role in the initial recognition of airway pathogens and the subsequent recruitment of immune cells, and particularly DCs. The contribution of DCs to host defense against airway pathogens and allergens is well-recognized (45). In addition to sampling antigens in the airway and communicating with T and B cells in local lymph nodes, pulmonary DCs coordinate the removal of apoptotic PMNs and DCs (24), a process important in the development of immunogenicity or tolerance. These DCs inform the T-cell response that is then appropriately polarized in response to the specific pathogens that are recognized (2).

Our data indicate that *P. aeruginosa* elicits a type I IFN response in the respiratory tract. The expected cell types, namely respiratory epithelial cells and DCs, were readily stimulated by *P. aeruginosa* to express IFN- β that directed the induction of STAT1 phosphorylation and the subsequent production of known effectors of this cascade, including IP-10, RANTES, CXCL-1, and IL-6. Using data obtained from well-characterized CF (Δ F508/W1282X) plus vector and corrected cell lines, we suggest that CFTR mutations may affect the epithelial induction of IFN- β expression by airway cells in response to *P. aeruginosa*, the most proximal step in the mucosal response to inhaled airway pathogens. This observation was confirmed in the CFBE41o⁻ cell line (Δ F508/ Δ F508), which stably expresses WT and Δ F508 CFTR. This was not a global defect in type I IFN signaling, which would not be expected, because patients with CF do not have clinical problems with typical viral infections, except as a cause for exacerbation of their bacterial infections. Instead, the defective signaling seems limited to TLR4/TRIF-mediated responses, which are important for the induction of IFN- β in response to LPS. A recent study indicated that this defect may be a consequence of decreased TLR4 expression in CF airway cells (46). It is unlikely to be coincidental that polymorphisms in TLR4 were associated with CF phenotypes (47). The TLR4-TRIF cascade is intracellular and localized to the endosome, whereas LPS/TLR4-MyD88 signaling (i.e., IL-8), which is initiated at the cell surface, was not diminished in the CF cells. The robust activation of IFN- β through a TLR3-dependent pathway, although displaying some reduction in transcription in the CFTR background, was still able to induce a significant amount of *Ifnb* compared with TLR4 signaling, and may perhaps provide a therapeutic target to enhance innate immune signaling in the lung.

In addition to IFN- β , we observed several other type I IFN-dependent gene products that were affected by CFTR mutations. These did not include genes thought to be influenza-specific such as PKR, but did include IP-10, a CXCR3 chemokine critically important in the development of a Th1 response to extracellular pathogens (48). Moreover, the endogenous levels of several type I IFN-dependent effectors were notably lower than those of the control C38 cell line, although they could have increased in response to stimulation with *P. aeruginosa* or LPS.

The signaling pathway activated by *P. aeruginosa* reflects the predominance of LPS as a major immunostimulant. Because LPS is shed by growing organisms, it is readily available for uptake by both immune and airway epithelial cells. With the exception of limited dependency upon CD14, *P. aeruginosa* stimulated a TLR4/TRIF/MD2/TANK binding kinase I-dependent induction of IFN- β . This result is consistent with the involvement of TRIF and IRF3 in the clearance of *P. aeruginosa* from the lung, as previously reported (18, 43). Although we did not identify other components of *P. aeruginosa* that might induce the expression of IFN- β , the type III secretion system was not involved, because mutants in various type III secretion system effectors were not impaired during the induction of IFN- β (data not shown). Given the dependence of IFN- β induction on TLR4/TRIF, we may reasonably conclude that LPS is the major effector of this cascade in the lung.

Exactly how an epithelial defect in IFN- β and likely other type I IFN-dependent gene products contributes to the development of chronic infection in CF remains to be established. PMNs were thought to be the critical component of CF airway disease and a potential target for therapy. However, we speculate that the much smaller populations of regulatory cells (i.e., the DCs and Th17 populations) must be appropriately activated early in the pathogenesis of CF lung disease, before the lung becomes tolerized to the presence of bacteria. Although we found no defects in the expression of DC cytokines, the failure to activate immature DCs appropriately is likely to be important in the early stages of bacterial clearance. In poly(I:C)-treated mice, a significant enhancement in bacterial clearance occurred that could only be correlated with the presence of an activated DC/macrophage (CD86⁺ and CD11c^{med/hi}) population, and not with absolute numbers of phagocytic cells. Our model of acute infection does not address what happens as bacterial infection persists, but does suggest that enhancing the function of DCs, even before infection, may act to prevent colonization.

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