Differential Responses to Rhinovirus- and Influenza-associated Pulmonary Exacerbations in Patients with Cystic Fibrosis

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Abstract

Rationale: The mechanism by which viruses cause exacerbations of chronic airway disease and the capacity of patients with cystic fibrosis (CF) to respond to viral infection are not precisely known.

Objectives: To determine the antiviral response to infection in patients with CF.

Methods: Sputum was collected from patients with CF with respiratory exacerbation. Viruses were detected in multiplex polymerase chain reaction (PCR)–based assays. Gene expression of 84 antiviral response genes was measured, using a focused quantitative PCR gene array.

Measurements and Main Results: We examined 36 samples from 23 patients with respiratory exacerbation. Fourteen samples tested virus-positive and 22 virus-negative. When we compared exacerbations associated with rhinovirus (RV, n = 9) and influenza (n = 5) with virus-negative specimens, we found distinct patterns of antiviral gene expression. RV was associated with greater than twofold induction of five genes, including those encoding the monocyte-attracting chemokines CXCL10, CXCL11, and CXCL9. Influenza was associated with overexpression of 20 genes, including those encoding the cytokines tumor necrosis factor and IL-12; the kinases MEK, TBK-1, and STAT-1; the apoptosis proteins caspase-8 and caspase-10; the influenza double-stranded RNA receptor RIG-I and its downstream effector MAVS; and pyrin, an IFN-stimulated protein involved in influenza resistance.

Conclusions: We conclude that virus-induced exacerbations of CF are associated with immune responses tailored to specific infections. Influenza induced a more potent response consisting of inflammation, whereas RV infection had a pronounced effect on chemokine expression. As far as we are aware, this study is the first to compare specific responses to different viruses in live patients with chronic airway disease.

Keywords: chemokine; interferon; pattern recognition receptor; RNA helicase; Toll-like receptor

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Increasing evidence suggests that upper respiratory viral infections play a role in exacerbation and progression of cystic fibrosis (CF) lung disease. Viruses have been detected in 40–52% of patients with pulmonary exacerbations compared with only 9–18% of patients in stable clinical condition (1–3). Viruses detected include rhinovirus (RV), influenza A/B, respiratory syncytial virus (RSV), parainfluenza, adenovirus and human metapneumovirus, with RV being the most common (1–7). Although the frequency of viral isolation is similar in normal healthy control subjects and patients with CF, the clinical impact of viral infection in patients with CF is higher than in control subjects (5, 8–10). Patients with virus-associated lower respiratory symptoms had a higher frequency of exacerbations and hospitalizations (5, 8, 9, 11–13), increased use of antibiotics at long-term follow-up (2, 4–6, 14), and had a shorter time to next pulmonary exacerbation (14). Viral infections also lead to deterioration in clinical status (3, 4, 6, 8, 10, 11). Children with CF hospitalized with severe respiratory symptoms were

statistically more likely to become colonized with *Pseudomonas aeruginosa* in the subsequent 12–60 months compared with nonhospitalized patients (15). In another study, five of six children acquired first *P. aeruginosa* infection during or 3 weeks after a viral upper respiratory tract infection (4). In older children already colonized with *P. aeruginosa*, viral infection caused severe exacerbations, increased bacterial load, and the appearance of anti-pseudomonal antibodies (16).

More recently, it has been suggested that patients with CF are particularly susceptible to viral infection. In one study, cultured CF airway epithelial cells allowed increased replication of parainfluenza virus and were deficient in the synthesis of nitric oxide and activation of signal transducers and activators of transcription (STAT)-1, a key serine/threonine kinase involved in the transduction of extracellular IFN signals, leading to the expression of IFN-stimulated genes (ISGs) (17). Subsequently, it was shown that bronchoalveolar lavage RV load was higher in children with CF undergoing clinically indicated bronchoscopy compared with children with asthma or control subjects. RV load was negatively associated with IFN-β level (18). IL-8 production is not impaired (18–20). On the other hand, a comparison of influenza-induced gene expression in normal and CF airway epithelial cells showed similar patterns of response in the IFN-γ/STAT-1–regulated genes (21).

In the present study, we examined sputum specimens from patients with CF with respiratory exacerbations, using quantitative polymerase chain reaction (PCR) to detect respiratory viral infection and to analyze expression of viral response genes. We hypothesized that patients with respiratory viral infection would demonstrate increases in the expression of ISGs. Also, on the basis of potential differences in the response of *in vivo* and cell culture systems to various respiratory viruses, we also sought additional information on the response of patients to specific viral infections.

Methods

Sample Selection

This was a retrospective, single-center study. Sputum samples from University of Michigan Hospital (Ann Arbor, MI) patients with CF were stored for various amounts of time at 4°C in the clinical laboratory after routine processing and then transferred to −80°C. Samples from the fall/winter seasons between September 2009 and April 2012 were identified. From this set, we chose samples collected during CF pulmonary exacerbations. A pulmonary exacerbation was defined as a clinical change, resulting in inpatient antibiotic treatment (22). This definition is consistent with a consensus report defining exacerbation as the necessity for antibiotics indicated by a change in clinical parameters (23). Samples were collected on the day of hospitalization or during the clinic visit before hospital admission. FEV_1 and body mass index (BMI) data were also collected at the beginning of each exacerbation. Relevant pulmonary symptoms included increase in cough, change in sputum production (volume and/or appearance), onset or increase in hemoptysis, increased shortness of breath, and/or decreased exercise tolerance. We excluded patients with CF who had undergone lung transplantation. We identified 36 such specimens. Sample collection and medical record review were approved by the University of Michigan Institutional Review Board.

RNA Processing and Viral Detection

RNA was extracted from a 400-μl aliquot of sputum, using a TRIzol–chloroform method, suspended in 60 μl of nuclease-free water (Promega, Madison, WI), and reverse-transcribed to complementary DNA (cDNA). Reverse transcription was performed with a MultiScribe high-capacity cDNA RT kit (Applied Biosystems, Foster City, CA).

Viruses were detected by two methods. First, we employed a Seeplex RV15 ACE detection kit (Seegene, Gaithersburg, MD). This multiplex assay simultaneously amplifies target sequences for 15 respiratory viruses (24): human parainfluenza viruses 1–4; metapneumovirus; coronaviruses 229E/NL63 and OC43; adenovirus; influenza viruses A and B; respiratory syncytial virus (RSV) A and B; rhinoviruses A, B, and C; enterovirus; and bocaviruses 1–4. Detection is by agarose gel electrophoresis. Second, we used a novel PCR/ligase detection reaction (LDR) multiplex assay that simultaneously amplifies species-specific genomic loci for 14 respiratory viruses (24). In this assay, two short synthetic DNA probes complementary to the target sequences are joined by DNA ligase. Detection is by fluorescence-labeled ligation oligonucleotides in capillary electrophoresis. This method detects influenza A and B; parainfluenza 1, 2, 3, 4A, and 4B; coronaviruses 229E and OC43; influenza A and B; rhinoviruses A, B, and C; adenoviruses A–E; metapneumovirus; and RSV A and B.

Quantitative PCR

TRIzol-purified RNA was further refined with an RNeasy MinElute cleanup kit (Qiagen, Valencia, CA). Because of low RNA yields for some samples, cleaned-up RNA was amplified with an RT^2 PreAMP cDNA synthesis kit (SABiosciences/Qiagen, Frederick, MD). Corresponding cDNA was added to RT^2 SYBR green master mix (SA Biosciences/Qiagen). Gene expression profiles were constructed by quantitative two-step real time PCR using the RT^2 profiler PCR array for human antiviral response genes (SABiosciences/Qiagen). This array contains 84 genes involved in the innate antiviral immune response, including the receptors and signaling effectors for pattern recognition receptors, the genes responsive to these pathways, and the genes involved in type-I IFN signaling as well as downstream IFN-stimulated genes.

Statistical Analysis

To analyze clinical data, we used unpaired *t* and Fisher exact tests where appropriate. To analyze focused gene array data, we used an online program from the manufacturer's website (http://www.sabiosciences.com/pcrarraydataanalysis.php). For each PCR, the program calculated two normalized average cycle threshold (Ct) values, a paired *t* test *P* value, and a fold change. Data normalization was based on correcting all Ct values for the average Ct values of constantly expressed housekeeping genes present on the array. The $2^{-\Delta\Delta Ct}$ method was used to calculate the fold change in the normalized Ct values. The online program does not adjust *P* values for multiple tests. We therefore corrected for multiple comparisons by calculating the Benjamini and Hochberg false discovery rate (25).

Results

We examined 36 sputum samples from 23 patients undergoing a CF respiratory

exacerbation. Fourteen samples from 13 patients tested virus-positive and 22 samples from 18 patients were virus-negative. Eight subjects contributed both virus-positive and virus-negative samples. Patient characteristics are shown in Table 1. The median age for all patients was 21.1 years (range, 12.0–36.6), and the median FEV_1 was 42% (range, 15–91). Fifty-eight percent of the samples were positive for *Pseudomonas aeruginosa*. Combining results from the two assays, the most common single viral infection was RV (n = 9) followed by influenza A (n = 5). The Seegene kit appeared to be more sensitive than PCR/LDR for the detection of RV (Table 2), likely because of the difficulty in finding a unique ligation probe for the numerous RV species. There was one coinfection with influenza A and parainfluenza 1. Patients infected with influenza had lower lung function and tended to be older than patients with RV (Table 1).

When we compared specimens from exacerbations associated with viral infection (n = 14) with specimens from virus-negative exacerbations (n = 22), we found that 8 of the 84 antiviral response genes on the focused gene array were up-regulated at least 2-fold, and the uncorrected *P* values of five additional genes were less than 0.05 (Table 3). Two genes encoded IFN-inducible chemokines (*CXCL10*, *CXCL11*). The *IL15* gene product induces an antiviral state through the regulation of type I IFN production and natural killer cell proliferation (26, 27). *AIM2* encodes an IFN-inducible cytosolic double-stranded DNA sensor (28). *SUGT1* encodes a Nod-like receptor protein 3–binding protein (29). *TLR8* encodes a membrane-bound receptor for single-stranded RNA. *AZI2* and *CHUK* encode kinases that regulate NF-κB signaling (30). *RELA* encodes the NF-κB p65 subunit. *TBK1* encodes TANK-binding kinase (TBK), an enzyme that phosphorylates IFN response factors (IRFs) mediating virus-induced type I IFN production (31). The *CYLD* gene product prevents activation of TBK, thereby inhibiting IFN production (32). mRNA levels for *IFNA1*, *IFNA2*, and *IFNB1* were nearly twofold higher in the virus-positive samples, but expression levels were low (average cycle numbers in the 33–34 range).

When we specifically compared specimens from exacerbations associated with RV infection (n = 9) with specimens from virus-negative exacerbations (n = 22), four genes were up-regulated at least twofold by viral infection, and the uncorrected *P* value of one additional gene was less than 0.05 (Table 4). Patients with RV showed greater than twofold expression of three genes encoding monocyte-attracting chemokines (*CXCL10*, *CXCL11*, and *CXCL9*). *IFIH1* encodes melanoma differentiation–associated protein (MDA)-5, an intracellular RNA helicase that recognizes RV double-stranded RNA. *IL15* (described previously) was also induced by RV infection.

When we specifically compared samples from exacerbations associated with influenza A-positive samples (n = 5) with virus-negative samples (n = 22), patients with influenza showed greater than 2-fold expression of 21 genes, and the up-regulation of 8 genes was statistically significant (Table 5). Many of these genes encode proteins involved

Table 1. Patient clinical characteristics*

	Virus (–)	Virus (+)	*P* Value
n, samples	22	14	
n, patients	18	13	
Age (yr), median (range)	21.3 (14.8–36.6)	19.8 (14.0–36.5)	0.987
FEV_1 (%), median (range)	45 (17–89)	34 (15–91)	0.706
BMI (kg/m^2), median (range)	20.1 (16.0–24.7)	20.6 (15.1–21.8)	0.626
Homozygous F508del: *n* (% of patients)	10 (56)	5 (38)	0.473
CFRD: *n* (% of samples)	9 (50)	8 (62)	0.717
Bacteria: *n* (% of samples)			
Pseudomonas aeruginosa	12 (55)	9 (64)	0.731
Staphylococcus aureus	1 (5)	1 (7)	1.000
Burkholderia cepacia complex	2 (9)	1 (7)	1.000
Burkholderia gladioli	1 (5)	2 (14)	0.547
Achromobacter sp.	2 (9)	0	0.511
Stenotrophomonas maltophilia	1 (5)	0	1.000
	Influenza (+)	**RV (+)**	***P* Value**
n, samples	5	9	
n, patients	5	9	
Age (yr), median (range)	26.7 (15.2–36.5)	19.4 (14–33.3)	0.232
FEV_1 (%), median (range)	32 (16–34)	65 (15–91)	0.009
BMI (kg/m^2), median (range)	17.0 (16.4–21.3)	20.6 (15.1–21.8)	0.812
Homozygous F508del: *n* (% of patients)	1 (20)	4 (44)	0.580
CFRD: *n* (% of samples)	4 (80)	4 (44)	0.301
Bacteria: *n* (% of samples)			
Pseudomonas aeruginosa	4 (80)	5 (55)	0.580
Staphylococcus aureus	0	1 (11)	1.000
Burkholderia cepacia complex	0	1 (11)	1.000
Burkholderia gladioli	1 (20)	1 (11)	1.000

Definition of abbreviations: BMI = body mass index; CFRD = cystic fibrosis–related diabetes; RV = rhinovirus.
*Eight subjects contributed both virus-positive and virus-negative samples; four subjects contributed two virus-negative samples and one subject contributed two virus-positive samples.

Table 2. Viruses detected in cystic fibrosis respiratory samples

	Method of Viral Detection	
Sample	**Seegene PCR**	**PCR/LDR**
1	RV	
2	RV	
3		Influenza A
4	Parainfluenza 1	Influenza A
5	RV	
6	Influenza A	Influenza A
7	Influenza A	Influenza A
8	RV	
9	RV	
10	Influenza A	Influenza A
11	RV	
12	RV	
13	RV	
14	RV	

Definition of abbreviations: PCR/LDR = polymerase chain reaction/ligase detection reaction; RV = rhinovirus.

Table 3. Gene overexpression in 14 virus-positive samples*

Gene Symbol	Protein	Fold Regulation	Uncorrected *P* Value	FDR
AIM2	Absent in melanoma 2	2.3982	0.127	0.183
AZI2	5-azacytidine induced 2	1.6875	0.020	0.086
CHUK	Conserved helix-loop-helix ubiquitous kinase	1.5727	0.018	0.119
CXCL10	C-X-C motif chemokine 10	2.2304	0.283	0.307
CXCL11	C-X-C motif chemokine 11	3.8478	0.286	0.286
CYLD	Cylindromatosis (turban tumor syndrome)	1.5886	0.015	0.202
IL15	IL-15	2.9136	0.115	0.215
PYCARD	PYD and CARD domain containing	1.3039	0.040	0.104
RELA	NF-κB p65 subunit	1.3122	0.024	0.078
SUGT1	Suppressor of G2 allele of SKP1 homolog	2.3812	0.124	0.201
TBK1	TANK-binding kinase 1	2.0739	0.139	0.181
TLR8	Toll-like receptor 8	2.1485	0.104	0.226
TRAF3	TNF receptor–associated factor 3	2.0071	0.155	0.183

Definition of abbreviations: FDR = false discovery rate; TNF = tumor necrosis factor.
*Compared with 22 virus-negative samples.

in inflammation, for example, the cytokines tumor necrosis factor (TNF)-α and IL-12. TRAF3 and TRAF6 associate with and mediate signal transduction from various TNF receptor superfamily members. IL-12 is a type 1 cytokine classically expressed after viral infections. *IKBKB* encodes IκB kinase-β, an activator of NF-κB signaling. *NFKBIA* encodes IκBα, which prevents NF-κB activation by masking nuclear localization signals. *IFNAR1* encodes the type I IFN receptor. *MEFV* encodes an IFN-inducible protein involved in the antiviral response. STAT-1 is a key serine/threonine kinase involved in the transduction of extracellular IFN signals, leading to the expression of ISGs. *DDX58* encodes retinoic acid–inducible gene (RIG)-I, the intracytoplasmic receptor responsible for recognition of influenza double-stranded RNA. The MAVS adaptor protein acts downstream of RIG-I to coordinate activation of NF-κB and IRFs. *CD80* encodes B7-1, a protein found on activated B cells and monocytes that provides a costimulatory signal necessary for T-cell activation and survival. *CASP8* and *CASP10* encode cysteine-aspartic acid proteases (caspases), which play a central role in the execution phase of cell apoptosis.

When we directly compared samples from exacerbations associated with RV and influenza infections, patients with influenza showed greater than 2-fold higher expression of 32 genes, and the up-regulation of 10 of these genes was statistically significant (Table 6). In addition to the genes described in the previous paragraph, influenza induced overexpression of a number of genes associated with the inflammatory/immune response. *NFKB1* encodes the 50-kD subunit of NF-κB. *SPP1* encodes osteopontin, a cytokine that promotes the release of IL-12 and IFN-γ and hence participates in the development of protective cell-mediated immunity (33). *TLR3* encodes the Toll-like receptor recognizing viral double-stranded RNA, and *TICAM1* encodes the TLR3 adaptor protein. *MYD88* encodes the adaptor proteins for other Toll-like receptors, including TLR8. *TRIM25* encodes a ubiquitin E3 ligase that mediates the activation of RIG-I (34).

MAP2K1, *MAP2K3*, *MAPK8*, and *MAPK14* encode *m*itogen-activated protein (MAP)/*e*xtracellular signal–regulated kinase *k*inase (MEK)-1, MAP kinase kinase 3 (p38 MAP kinase kinase), Jun N-terminal kinase (JNK), and p38 MAP kinase, serine/threonine kinases regulating activation of the activator protein (AP)-1 family transcription factors, including the c-Jun proto-oncogene. Like *MEFV*, *MX1* is an ISG involved in influenza resistance. Cathepsin B is a lysosomal cysteine proteinase that is induced by influenza infection (35) and binds to the influenza nonstructural protein NS1 (36). Finally, compared with patients with influenza, patients with RV had greater than twofold increases in the chemokines encoded by *CXCL10*, *CXCL11*, *CXCL9*, as well as *IFNB1* and *TLR9*.

Discussion

We examined sputum samples from patients with CF undergoing a respiratory exacerbation. Compared with virus-negative samples, virus-positive samples showed

Table 4. Gene overexpression in nine rhinovirus-positive samples*

Gene Symbol	Protein	Fold Regulation	Uncorrected *P* Value	FDR
AIM2	Absent in melanoma 2	2.15	0.527	0.632
CXCL10	C-X-C motif chemokine 10	7.430	0.024	0.074
CXCL11	C-X-C motif chemokine 11	5.943	0.019	0.118
IFIH1	Melanoma differentiation–associated protein-5	1.6739	0.042	0.085
IL15	IL-15	2.3857	0.634	0.634

Definition of abbreviation: FDR = false discovery rate.
*Compared with 22 virus-negative samples.

Table 5. Gene overexpression in five influenza-positive samples*

Gene Symbol	Protein	Fold Regulation	Uncorrected *P* Value	FDR
AIM2	Absent in melanoma 2	2.024	0.514	0.581
AZI2	5-azacytidine induced 2	1.8315	0.003	0.077
CASP10	Caspase 10	1.9982	0.020	0.107
CASP8	Caspase 8	2.2626	0.115	0.230
CD80	CD80/B7-1	2.6996	0.980	0.980
DDX58	Retinoic acid–inducible gene-I	2.0457	0.611	0.635
HSP90AA1	Heat shock protein 90kDa α, class A member	11.8471	0.027	0.103
IFNAR1	Interferon-α/β receptor α chain	2.0322	0.156	0.270
IKBKB	Inhibitor of κ light polypeptide gene enhancer in B cells, kinase β (IκB kinase-β)	1.5971	0.025	0.111
IL12A	IL-12A	2.7291	0.367	0.434
IL12B	IL-12B	3.6472	0.358	0.444
IL15	IL-15	3.1802	0.185	0.300
IRAK1	IL-1 receptor–associated kinase 1	2.2494	0.069	0.391
IRF5	Interferon regulatory factor 3	2.0123	0.328	0.163
MAP2K1	Mitogen-activated protein kinase kinase 1 (MEK)	4.7158	0.009	0.427
MAVS	Mitochondrial antiviral signaling protein	2.355	0.010	0.129
MEFV	Pyrin	1.9129	0.041	0.094
NFKBIA	NF-κB inhibitor, α (IκBα)	2.5905	0.056	0.136
STAT1	Signal transducer and activator of transcription-1	12.3191	0.568	0.615
SUGT1	Suppressor of G2 allele of SKP1 homolog	2.2224	0.306	0.419
TBK1	TANK-binding kinase 1	3.685	0.015	0.098
TLR8	Toll-like receptor 8	2.931	0.143	0.267
TNF	Tumor necrosis factor-α	2.6749	0.263	0.403
TRAF3	TNF receptor–associated factor 3	3.1544	0.052	0.151
TRAF6	TNF receptor–associated factor 6	2.2136	0.112	0.244

Definition of abbreviations: FDR = false discovery rate; TNF = tumor necrosis factor.
*Compared with 22 virus-negative samples.

increased expression of host genes related to an antiviral response, including those encoding cytokines, chemokines, ISGs, and signaling molecules related to inflammation and apoptosis. These data suggest that patients with CF do indeed generate an antiviral response. Because we do not have a control group of respiratory samples from unaffected individuals, we cannot say whether this response is entirely appropriate. Nevertheless, our data provide insight into the responses of patients with CF to RV and influenza infection.

When we specifically compared exacerbations associated with RV with nonviral exacerbations (Table 4), patients with RV showed greater than twofold greater expression of three chemokine genes (*CXCL10*, *CXCL11*, and *CXCL9*). CXCL10 (also called IFN-γ–inducible protein 10), a chemoattractant for activated type 1 T lymphocytes and natural killer cells, has been previously demonstrated to be a biomarker of RV infection (37–41). Like CXCL10, CXCL11 and CXCL9 are strongly induced by IFNs, chemotactic for activated T cells, and highly expressed after RV infection (42). We also found significant overexpression of the *IFIH1* gene encoding MDA-5, an intracellular RNA helicase that recognizes viral double-stranded RNA. We have shown that, in contrast to RIG-I, MDA-5 expression is increased on RV infection in airway epithelial cells and is required for maximal RV-induced cytokine and IFN production in both human airway epithelial cells (43) and mouse lungs (44). Together, these data demonstrate that patients with CF with RV-associated respiratory exacerbations generate an antiviral response similar in character to that observed in experimental systems.

When we specifically compared exacerbations associated with influenza with virus-negative exacerbations (Table 5), patients with influenza showed more than twofold greater expression of many genes encoding proinflammatory cytokines and signaling intermediates. In contrast to RV, which induced expression of chemokines for mononuclear cells, influenza induced the cytokine TNF-α and its downstream effectors TRAF3 and TRAF6, which collectively stimulate systemic inflammation. *MAP2K1*, *MAP2K3*, *MAP3K7*, *MAPK8*, *MAPK14*, and *TBK1* encode the signaling intermediates MEK-1, p38 MAP kinase kinase, p38 MAP kinase kinase kinase, JNK, p38 MAP kinase, and TANK-binding kinase-1, respectively, each of which targets transcription factors necessary for virus-induced cytokine gene expression. *MX1* is an ISG involved in antiviral resistance against influenza viruses, which requires type I or type III IFN for induction (45). The observed increase in *STAT-1* is consistent with the normal response of cultured CF airway epithelial cells to influenza infection (21). Together, these data suggest that influenza infection of patients with CF induces a qualitatively and quantitatively different antiviral response than RV infection, one that is specifically tailored toward influenza virus and organized to produce a more robust inflammatory response, one that includes systemic manifestations and cell death.

The precise mechanism for reduced airway IFN production in patients with CF, if present, is not completely clear. An examination of BAL fluid from patients with CF showed a helper T-cell type 2 (Th2)- and Th17-dominated cytokine/chemokine profile (46). IL-17A, IL-1β, IL-6, IL-13, IL-5, and IFN-γ were

Table 6. Differences in host antiviral gene expression between influenza-positive samples and rhinovirus-positive samples

Gene Symbol	Protein	Fold Regulation	Uncorrected *P* Value	FDR
CCL3	C-X-C motif chemokine 3	2.8761	0.032	0.134
CD40	CD40, TNF receptor superfamily member 5	2.713	0.108	0.236
CD80	CD80/B7-1	3.7566	0.154	0.302
CTSB	Cathepsin B	2.3239	0.024	0.112
CXCL10	C-X-C motif chemokine 10	−26.568	0.093	0,216
CXCL11	C-X-C motif chemokine 11	−3.0398	0.468	0.577
CXCL9	C-X-C motif chemokine 9	−3.7826	0.234	0.361
DDX58	Retinoic acid–inducible gene-I	2.8931	0.492	0.588
HSP90AA1	Heat shock protein 90kDa α, class A member	2.2825	0.079	0.195
IFNAR1	Interferon-α/β receptor α chain	4.4748	0.415	0.548
IFNB1	Interferon, β1, fibroblast	−2.2168	0.439	0.561
IL12A	IL-12A	7.7172	0.121	0.250
IL12B	IL-12B	8.0161	0.002	0.041
IRAK1	IL-1 receptor–associated kinase 1	2.6623	0.004	0.049
IRF5	Interferon regulatory factor 5	3.3067	0.305	0.419
JUN	Jun proto-oncogene	2.0744	0.013	0.085
MAP2K1	Mitogen-activated protein kinase kinase 1 (MEK)	8.6054	0.007	0.070
MAP2K3	Mitogen-activated protein kinase kinase 3 (MKK3)	2.0332	0.209	0.368
MAPK14	Mitogen-activated protein kinase 14 (p38 MAPK)	2.0152	0.717	0.780
MAPK8	Mitogen-activated protein kinase 8/Jun N-terminal kinase (JNK)	3.3135	0.573	0.663
MAVS	Mitochondrial antiviral signaling protein	6.0483	0.063	0.181
MEFV	Pyrin	7.5083	0.224	0.378
MX1	Myxovirus (influenza virus) resistance 1	12.702	0.603	0.676
MYD88	Myeloid differentiation primary response gene (88)	5.4022	0.200	0.371
NFKB1	Nuclear factor of κ light polypeptide gene enhancer in B-cells-1/NF-κB p50	2.9631	0.018	0.095
NFKBIA	NF-κB inhibitor, α (IκBα)	2.0554	0.074	0.197
PYDC1	PYD (pyrin domain) containing 1	2.1224	0.230	0.370
SPP1	Secreted phosphoprotein 1/osteopontin	5.0982	0.984	0.984
STAT1	Signal transducer and activator of transcription-1	13.6147	0.303	0.431
TBK1	TANK-binding kinase-1	3.2648	0.039	0.146
TICAM1	Toll-like receptor adaptor molecule 1	2.5642	<0.001	0.010
TLR8	Toll-like receptor 8	2.1646	0.260	0.385
TLR9	Toll-like receptor 9	−2.7326	0.861	0.910
TNF	Tumor necrosis factor-α	5.4128	0.051	0.174
TRAF3	TNF receptor-associated factor 3	2.6977	0.008	0.065
TRAF6	TNF receptor-associated factor 6	2.4659	0.052	0.160
TRIM25	Tripartite motif containing 25	3.2713	0.901	0.926

Definition of abbreviations: FDR = false discovery rate; TNF = tumor necrosis factor.

significantly higher in the BAL fluid of patients with CF compared with non-CF control subjects, and levels were even higher in patients with signs of pulmonary exacerbation. Because the differentiation of Th1 and Th2 cell lineages is mutually antagonistic (e.g., IL-4 blocks Th1 differentiation) (47), patients with CF and Th2/Th17 polarization may have a deficiency in their IFN response. In addition, prior infection with *Pseudomonas aeruginosa* has been shown to suppress IFN responses to subsequent viral infection in CF bronchial epithelial cells (48).

There are several potential limitations to our study. First, in our analysis, we included genes that were up-regulated greater than twofold but not statistically significant. Although examining the fold increase in gene expression lacks a solid statistical footing, examining fold increase data is the simplest and most intuitive approach to finding genes that are differentially regulated between control and experiment. Also, statistical methods that correct for the number of comparisons may inadvertently reduce the power of analysis, significantly altering microarray interpretations so that no conclusions can be reached (49). This is particularly a problem when the number of samples is low. Fold increase data may also be useful for hypothesis generation which can be followed up with specific gene expression studies in future experiments/cohorts. Second, as noted previously, we did not study a control group of respiratory samples from unaffected individuals; therefore, we cannot say whether the CF antiviral response was appropriate. Third, because we studied sputum samples, we cannot comment on the cellular source of the measured mRNAs. CF sputum contains a mixture of squamous cells, respiratory epithelial cells, and inflammatory cells, with the majority being neutrophils. Although the respiratory epithelium is a major target of respiratory viruses, monocytes/macrophages may also be infected (50, 51). Fourth, because of limited sample volumes, we did not attempt to confirm translation of the various sputum mRNAs into protein.

We conclude that patients with CF generate a significant innate immune response to respiratory viral infections.

RV is associated with a modest response characterized by mRNAs encoding chemokines and the RV double-stranded RNA receptor MDA5. Influenza infection is associated with a distinctly different response, inducing mRNAs that promote cytokine production, systemic inflammation, cell death, and specific antiviral proteins. Further insight into these antiviral responses could lead to therapeutic interventions against virus-induced CF exacerbations. ■

Author disclosures are available with the text of this perspective at www.atsjournals.org.

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