

## ORIGINAL ARTICLE

# Impaired type I and type III interferon induction and rhinovirus control in human cystic fibrosis airway epithelial cells

Marjolaine Vareille,<sup>1,2,3,4</sup> Elisabeth Kieninger,<sup>1,4</sup> Marco P Alves,<sup>1,4</sup> Brigitte S Kopf,<sup>1,4</sup> Alexander Möller,<sup>5</sup> Thomas Geiser,<sup>6</sup> Sebastian L Johnston,<sup>7</sup> Michael R Edwards,<sup>7</sup> Nicolas Regamey<sup>1,4</sup>

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<sup>1</sup>Department of Clinical Research, University of Bern, Bern, Switzerland

<sup>2</sup>Institute for Infectious Diseases, University of Bern, Bern, Switzerland

<sup>3</sup>Laboratoire d'Immunologie, Facultés de Médecine et Pharmacie, Clermont-Ferrand, France

<sup>4</sup>Division of Respiratory Medicine, Department of Paediatrics, Inselspital and University of Bern, Bern, Switzerland

<sup>5</sup>Department of Respiratory Medicine, University Children's Hospital, Zürich, Switzerland

<sup>6</sup>Division of Respiratory Medicine, University Hospital of Bern, Inselspital, Bern, Switzerland

<sup>7</sup>Department of Respiratory Medicine, National Heart and Lung Institute, Wright Fleming Institute of Infection and Immunity & MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, Imperial College London, London, UK

## Correspondence to

Professor Nicolas Regamey, Division of Respiratory Medicine, Department of Paediatrics, University Children's Hospital of Bern, Inselspital, Bern 3010, Switzerland; [nicolas.regamey@insel.ch](mailto:nicolas.regamey@insel.ch)

MV and EK contributed equally.

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## ABSTRACT

**Background** Rhinoviruses are important triggers of pulmonary exacerbations and possible contributors to long-term respiratory morbidity in cystic fibrosis (CF), but mechanisms leading to rhinovirus-induced CF exacerbations are poorly understood. It is hypothesised that there is a deficient innate immune response of the airway epithelium towards rhinovirus infection in CF.

**Methods** Early innate immune responses towards rhinoviruses (RV-16, major-type and RV-1B, minor-type) in CF and non-CF bronchial epithelial cell lines and primary nasal and bronchial epithelial cells from patients with CF (n=13) and healthy controls (n=24) were studied.

**Results** Rhinovirus RNA expression and virus release into supernatants was increased more than tenfold in CF cells compared with controls. CF cells expressed up to 1000 times less interferon (IFN) type I ( $\beta$ ) and type III ( $\lambda$ ) mRNA and produced less than half of IFN- $\beta$  and IFN- $\lambda$  protein compared with controls. In contrast, interleukin 8 production was not impaired, indicating a selective deficiency in the innate antiviral defence system. Deficient IFN production was paralleled by lower expression of IFN-stimulated genes including myxovirus resistance A, 2',5'-oligoadenylate synthetase, viperin and nitric oxide synthase 2. Addition of exogenous type I and III IFNs, particularly IFN- $\beta$ , restored antiviral pathways and virus control in CF cells, underscoring the crucial role of these molecules.

**Conclusions** This study describes a novel mechanism to explain the increased susceptibility of patients with CF to rhinovirus infections. A profound impairment of the antiviral early innate response in CF airway epithelial cells was identified, suggesting a potential use of IFNs in the treatment of rhinovirus-induced CF exacerbations.

## INTRODUCTION

Respiratory virus infections have a significant impact on patients with cystic fibrosis (CF).<sup>1–3</sup> They have been linked to increased respiratory symptoms,<sup>2</sup> antibiotic use,<sup>4</sup> prolonged hospitalisations<sup>1</sup> and pulmonary function deterioration in both children and adults with CF.<sup>3,5</sup> Up to 40% of pulmonary exacerbations in CF and half of the hospital admissions of infants with CF are associated with respiratory viruses.<sup>1,3,6</sup> Rhinoviruses

## Key messages

### What is the key question?

► What are the mechanisms leading to rhinovirus-induced exacerbations in cystic fibrosis (CF)?

### What is the bottom line?

► Deficient virus control by the CF airway epithelium may explain the increased susceptibility of patients with CF towards rhinoviruses.

### Why read on?

► This study identifies defective interferon type I and III production as a key pathophysiological mechanism leading to impaired virus control by the CF airway epithelium.

(RVs), the agents of the common cold, have been shown to precipitate the majority of asthma exacerbations<sup>7</sup> and nearly half the exacerbations of chronic obstructive pulmonary disease (COPD).<sup>8</sup> RVs have consistently been identified as the predominant agents during virus-associated pulmonary exacerbations in CF.<sup>9,10</sup>

The airway epithelium is central to innate immune responses in the lung, which are crucial in the defence against respiratory viruses.<sup>11</sup> Interaction between respiratory viruses and airway epithelial cells (AECs) results in production of antiviral substances including type I ( $\alpha$  and  $\beta$ ) and III ( $\lambda$ ) interferons (IFNs) which contribute to virus clearance.<sup>12,13</sup> IFN- $\beta$  and IFN- $\lambda$  (IFN- $\lambda$ 1/IL-29, IFN- $\lambda$ 2/IL-28A and IFN- $\lambda$ 3/IL-28B) are the major IFNs produced by bronchial epithelial cells upon virus infection.<sup>14</sup> After binding to their specific receptors, they send a signal to the nucleus through the Jak-STAT pathway to induce IFN-stimulated genes (ISGs) leading to the production of several antiviral proteins. These include 2',5'-oligoadenylate synthetase (2',5'-OAS), myxovirus resistance (Mx) proteins, viperin and nitric oxide synthase (NOS) 2, which mediate the antiviral actions of IFNs by inhibiting virus replication.<sup>11</sup>

Recently, deficient induction of type I and III IFNs in bronchial epithelial cells from patients with asthma upon RV infection has been proposed as a mechanism to explain the susceptibility of

these patients towards RVs.<sup>15 16</sup> We hypothesised that similar deficiencies may be found in CF, and studied the early innate antiviral immune response of CF AECs towards RVs. In this paper we report increased RV replication due to deficient epithelial type I and III IFN production as a novel mechanism to explain the increased susceptibility of patients with CF to RV infections, and thus identify type I and III IFNs as potential treatments for virus-induced exacerbations in CF.

## METHODS

### Cell and virus culture

Three human bronchial epithelial cell lines with CF and non-CF phenotypes were used: UNCCF2T (F508del/F508del cystic fibrosis transmembrane conductance regulator (CFTR) mutation)/UNCN2T,<sup>17</sup> CFBE41o- (F508del/F508del CFTR mutation)/16HBE14o-<sup>18 19</sup> (both kindly donated by Drs S Randell and D C Gruenert) and IB3-1 (F508del/W1282X CFTR mutation)/IB3-S9.<sup>20</sup> Cells were grown as previously described.<sup>21</sup> Prior to infection, cells were seeded in 12-well tissue culture plates (Nunc, Rochester, USA) and placed into culture medium without supplements for 24 h. RV16 and RV1B stocks were grown in Ohio HeLa cells (European Collection of Cell Cultures) and stocks prepared as HeLa lysates at  $1 \times 10^7$  TCID<sub>50</sub>/ml.<sup>22</sup>

### Primary human airway epithelial cells

Primary human AECs obtained from patients with CF and healthy subjects were grown in Bronchial Epithelial Growth Medium (Lonza, Switzerland) according to the manufacturer's recommendations, as described previously.<sup>21</sup> At passage 2, cells were seeded onto 12-well plates until 80–90% confluency and grown without supplements for 24 h prior to infection.

### Virus infection

Cells were infected with RV16 or RV1B at a multiplicity of infection (MOI) of 2 for 1 h at room temperature with shaking. Virus preparations were removed, cells washed and 1 ml of fresh medium added. As negative controls, cells were treated with medium alone or filtered virus.<sup>20</sup> Plates were incubated at 37°C in 5% CO<sub>2</sub>. Cell lysates and supernatants were harvested at various time points and stored at –80°C.

### RT-qPCR

Quantitative RT-qPCR was carried out using specific primers and probes for RV, IFN- $\lambda$ 1, IFN- $\lambda$ 2/3, IFN- $\beta$ , interleukin (IL)-8, MxA, 2',5'-OAS, viperin, NOS2, Toll-like receptor 3 (TLR3), melanoma differentiation-associated gene 5 (MDA5), retinoic acid inducible gene I (RIG-I) and 18S (see table E1 in online supplement). Reactions were performed on iCycler (Biorad, USA). Gene expression was normalised to 18S rRNA<sup>23</sup> and expressed as copies per  $\mu$ g of total RNA.

### Measurement of virus particle release

Supernatants were serially diluted in Dulbecco's Modified Eagle Medium containing 4% FCS (Invitrogen, USA) and titrated on HeLa cells to determine the TCID<sub>50</sub>/ml of RV in the supernatants as described previously.<sup>24</sup>

### ELISA

Protein levels of IL-8, IFN- $\beta$  and IFN- $\lambda$ <sub>f</sub> (IFN- $\lambda$ 1/IL-29, IFN- $\lambda$ 3/IL-28B) were quantified in supernatants from untreated and infected cell cultures using ELISA kits for human IL-8

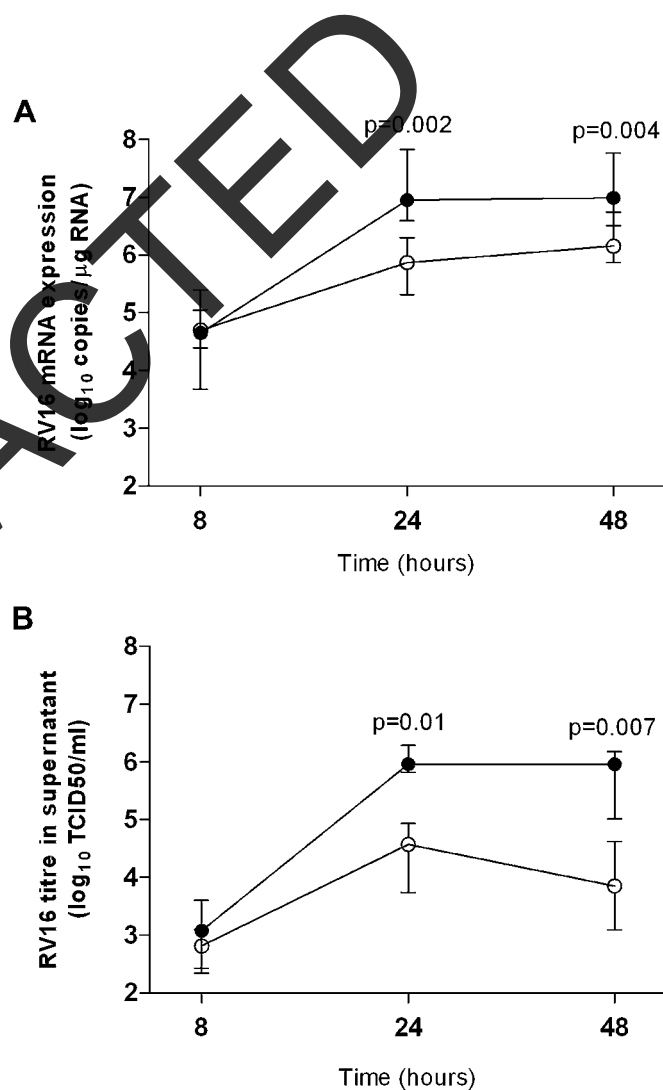
(Amersham Biosciences, USA), IFN- $\beta$  (Biosource International, USA) and IFN- $\lambda$ <sub>f</sub> (R&D Systems, USA).

### Biological effect of IFN- $\beta$ and IFN- $\lambda$

Cells were seeded and treated with/without different doses of IFN- $\beta$  and/or IFN- $\lambda$ 1 before and after infection (Peprotech, USA).

### Transfection and RNA interference

Healthy primary nasal AECs were transfected with 100 pmol of scrambled control or three different CFTR siRNAs by using lipofectamin 2000 (Invitrogen, USA) according to the manufacturer's instructions. siRNA sequences<sup>25</sup> and CFTR sequences of primers/probes are given in the online supplement and in supplemental table E1.



**Figure 1** Time course of rhinovirus (RV) replication and release in cystic fibrosis (CF) bronchial epithelial cells. (A) RV16 expression was measured by RT-qPCR after 8 h, 24 h and 48 h of infection (UNCCF2T/UNCN2T cells). Virus replication increased over time and was significantly higher in CF cells (closed circles) than in non-CF cells (open circles) at both 24 h and 48 h. (B) RV16 release into the supernatants of infected cells was determined by calculating the TCID<sub>50</sub>/ml by titration assay on Ohio HeLa cells. Virus load was significantly increased in CF compared with non-CF cells at both 24 h and 48 h. TCID<sub>50</sub>, 50% tissue culture infective dose. Data are presented as median (IQR) of 3–6 independent experiments.

**Table 1** Rhinovirus (RV) replication and release in cystic fibrosis (CF) and non-CF bronchial epithelial cells

	Control cells	CF cells	p Value
UNCN2T/UNCCF2T			
RV16 RNA expression (copies/ $\mu$ g RNA)	7.4 (2.1–19.8) $\times 10^5$	88.2 (39.0–6735.0) $\times 10^5$	0.002
RV16 titre (TCID <sub>50</sub> /ml)	0.3 (0.05–0.9) $\times 10^5$	9.2 (6.7–19.5) $\times 10^5$	0.01
RV1B RNA expression (copies/ $\mu$ g RNA)	4.9 (1.9–42.7) $\times 10^5$	164.5 (117.8–430.3) $\times 10^5$	0.03
RV1B titre (TCID <sub>50</sub> /ml)	0.01 (0.004–0.09) $\times 10^5$	0.2 (0.1–20.0) $\times 10^5$	0.03
IBS3-S9/IB3-1			
RV16 RNA expression (copies/ $\mu$ g RNA)	67.5 (30.7–85.9) $\times 10^5$	158.5 (50.2–470.8) $\times 10^5$	0.03
RV16 titre (TCID <sub>50</sub> /ml)	0.1 (0.02–0.5) $\times 10^5$	4.5 (1.3–8.4) $\times 10^5$	0.002
RV1B RNA expression (copies/ $\mu$ g RNA)	38.1 (4.3–82.0) $\times 10^5$	83.9 (17.9–188.8) $\times 10^5$	0.02
RV1B titre (TCID <sub>50</sub> /ml)	0.01 (0.01–0.01) $\times 10^5$	0.08 (0.02–0.6) $\times 10^5$	0.06
16HBE14o-/CFBE41o-			
RV16 RNA expression (copies/ $\mu$ g RNA)	18.9 (10.7–57.4) $\times 10^5$	926.5 (289.7–1747.05) $\times 10^5$	0.02
RV16 titre (TCID <sub>50</sub> /ml)	0.03 (0.01–0.2) $\times 10^5$	1.0 (0.3–5.7) $\times 10^5$	0.001
RV1B RNA expression (copies/ $\mu$ g RNA)	0.7 (0.2–1.4) $\times 10^5$	61.3 (19.7–93.0) $\times 10^5$	0.001
RV1B titre (TCID <sub>50</sub> /ml)	0.3 (0.06–0.9) $\times 10^5$	5.5 (0.6–9.0) $\times 10^5$	0.03

Data are presented as median (IQR). Cells were infected with RV16 or RV1B. RV RNA expression was analysed by RT-qPCR 24 h after infection. RV particle release was measured in culture supernatants 24 h after infection. TCID<sub>50</sub>, 50% tissue culture infective dose.

## Statistics

Data are presented as median (IQR). The Mann–Whitney U test was used to determine differences between groups. Associations were tested by univariable and multivariable regression analyses.

Additional details are provided in the online supplement.

## RESULTS

### Increased virus replication and release in CF bronchial epithelial cells

To investigate whether CF cells were more susceptible to RV infection than non-CF cells, we analysed RV16 RNA expression at 8, 24 and 48 h after infection. We found a more than tenfold increase of vRNA expression in CF cells (UNCCF2T) compared with non-CF cells (UNCN2T) (figure 1A). These results were confirmed by measuring virus particle release into supernatants (figure 1B). Experiments performed with the minor-group RV1B gave similar results (table 1), but replication was lower than for the major-group RV16 as observed by others.<sup>14–26</sup> The results were replicated in two other CF cell lines (table 1).

### Impaired IFN induction in CF bronchial epithelial cells

Type I ( $\alpha$ , $\beta$ ) and III ( $\lambda$ 1,  $\lambda$ 2/3) IFNs have been described as having strong antiviral properties against RVs.<sup>14</sup> We tested the hypothesis that increased virus replication in CF cells may be attributable to deficient IFN induction. IFN- $\beta$ , - $\lambda$ 1 and - $\lambda$ 2/3 gene expression was upregulated after infection in both CF and non-CF but was significantly impaired in CF (figure 2A–C). IFN- $\alpha$  mRNA was poorly expressed and not upregulated.<sup>14</sup> In accordance with mRNA data, IFN- $\beta$  and IFN- $\lambda$  protein production was increased in CF and control cells upon RV infection but impaired in CF, both at 24 h and 48 h (figure 2D,E).

### Specific IFN deficiency in CF bronchial epithelial cells

We assessed whether IFN production deficiency in CF cells was specific and not due to a global downregulation of gene expression and protein production by analysing induction of the proinflammatory cytokine IL-8. IL-8 gene expression was not

upregulated after infection in CF or control cells (figure 2F), but similar large amounts of IL-8 protein were secreted at both 24 h and 48 h after infection in CF and non-CF cells (figure 2G).

### Deficient induction of IFN-stimulated genes in CF bronchial epithelial cells

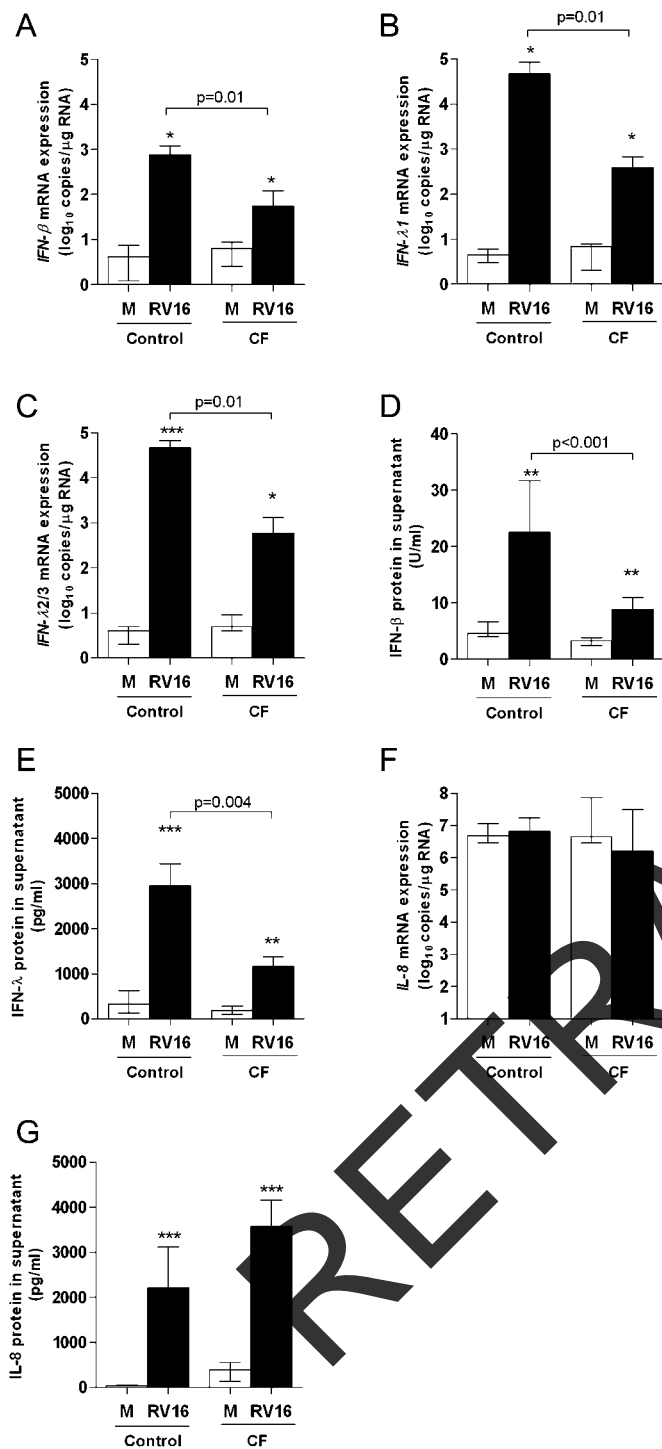
To study the downstream effects of impaired IFN induction in CF, we investigated the expression of MxA, 2',5'-OAS, viperin and NOS2. Expression of all ISGs was induced in CF and non-CF cells upon infection, but CF cells expressed up to 1000 times less ISG mRNA (figure 3A). ISG expression was inversely related to RV replication (2',5'-OAS:  $R^2=0.52$ ,  $p=0.005$ ; viperin:  $R^2=0.12$ ,  $p=0.142$ ; NOS2:  $R^2=0.29$ ,  $p=0.042$ ) and positively to IFN- $\beta$  production (2',5'-OAS:  $R^2=0.61$ ,  $p=0.002$ ; viperin:  $R^2=0.16$ ,  $p=0.106$ ; NOS2:  $R^2=0.39$ ,  $p=0.021$ ) (data for MxA shown in figure 3B,C). A similar positive relationship was found between ISG expression and IFN- $\lambda$  production (not shown). Associations remained significant after adjusting for disease group in multivariable regression analysis.

### Exogenous IFN restores virus control and decreases virus-induced cytotoxicity in bronchial epithelial CF cells

Having demonstrated increased virus replication and a selective IFN production deficiency in CF after RV infection, we tested the ability of exogenous IFN- $\beta$ /IFN- $\lambda$  to restore antiviral pathways. Both exogenous IFN- $\beta$  and IFN- $\lambda$  (100 pg/ml) increased levels of ISGs (figure 4A) and decreased RV16 RNA expression and release into supernatants of CF cells (figure 4B,C) to the level of control cells. IFN- $\beta$  had a more pronounced effect than IFN- $\lambda$ . No additional effect was observed when CF cells were treated with IFN- $\beta$  and IFN- $\lambda$  together. There was no effect of IFN treatment in control cells. Additionally, previously reported increased cytotoxicity upon RV infection in CF cells<sup>21</sup> could be decreased to the level of control cells after exogenous IFN treatment (see figure E1 in online supplement).

### Impaired virus control and IFN induction in primary CF airway epithelial cells

To assess whether our findings in cell lines could be replicated in primary AECs, we investigated virus replication in nasal and



**Figure 2** Impaired interferon (IFN)-β and IFN-λ induction in cystic fibrosis (CF) bronchial epithelial cells after rhinovirus (RV) infection. IFN and interleukin (IL)-8 mRNA expression were assessed by RT-qPCR in UNCCF2T/UNCN2T cells. RV16 induced the expression of genes encoding (A) IFN-β, (B) IFN-λ1 and (C) IFN-λ2/3 at 8 h after infection in both CF and non-CF cells (\* $p < 0.05$ , \*\*\* $p < 0.001$  vs medium control (M)). However, induction of both IFN-β and IFN-λ gene expression was significantly impaired in CF compared with non-CF cells (A–C). IL-8 gene expression did not increase in CF or in non-CF cells (F). Production of (D) IFN-β, (E) IFN-λ and (G) IL-8 protein in UNCCF2T/UNCN2T cell supernatants was increased in both CF and non-CF cells 48 h after RV16 infection (\*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs M). Production of IFN-β and IFN-λ protein was significantly impaired in CF cells compared with non-CF cells (D,E). In contrast, levels of IL-8 protein production were similar in CF and control cells (G). Data are presented as median (IQR) of 4–6 independent experiments.

bronchial AECs obtained from patients with CF and healthy subjects at a median (IQR) age of 15.1 (9.6–26.7) years (table 2). We found impaired control of RV replication in CF, with the virus load being 100-fold higher in nasal CF AECs and tenfold higher in bronchial CF AECs than in non-CF cells (figure 5A,B). IFN-β mRNA expression was 100 times lower in nasal CF AECs and 10 times lower in bronchial CF AECs compared with controls (figure 5C,D). Similar findings were obtained for IFN-β protein both at 24 h and 48 h (figure 5E,F). There was no association between virus load or IFN production and clinical markers in patients with CF (age, forced expiratory volume in 1 s, *Pseudomonas aeruginosa* colonisation). However, there was an inverse relationship between IFN-β levels and RV replication in nasal AECs ( $R^2=0.59$ ,  $p < 0.001$ ) and bronchial AECs ( $R^2=0.29$ ,  $p=0.03$ ; figure 6) which remained significant after adjusting for disease group in multivariable regression analysis.

As pattern recognition receptors (PRRs) including TLR3, MDA5 and RIG-I play a central role in RV recognition and virus-induced IFN responses,<sup>27,28</sup> we investigated whether expression of these receptors differed in CF compared with non-CF. Basal expression of all receptors was similar in CF and non-CF (figure E2 in online supplement).

#### Increased virus replication and impaired IFN responses are not directly linked to CFTR dysfunction

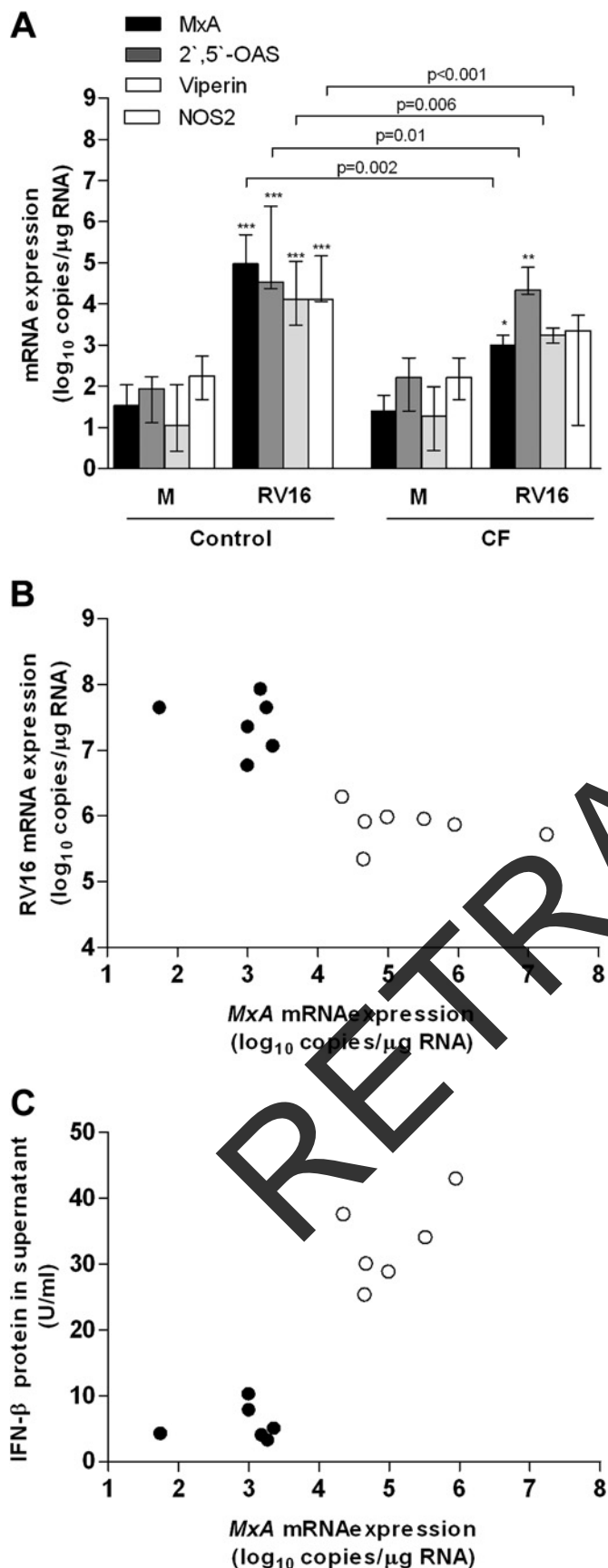
We examined RV replication and IFN responses after CFTR inhibition in healthy primary AECs. Upon transfection of siRNA targeting CFTR, levels of CFTR mRNA were decreased up to 80% in control and RV16-infected cells (figure 7A). However, there were no differences in virus replication or in IFN mRNA levels between control and CFTR siRNA-transfected cells (figure 7B, C).

#### DISCUSSION

Strategies aimed at controlling respiratory virus infections may limit exacerbations and deterioration of pulmonary function in patients with CF. Approaches targeting respiratory viruses in CF have included yearly immunisations,<sup>29</sup> the use of neuraminidase inhibitors or adamantanes<sup>30</sup> against influenza and prophylactic administration of immunoglobulins against respiratory syncytial virus.<sup>31</sup> However, no treatment against rhinoviruses is currently available. Impaired ISG production linked to impaired STAT1 activation or function of NOS2 was previously identified in CF ACEs grown from lung explants stimulated with para-influenza virus<sup>32–33</sup> and influenza virus.<sup>34</sup> We extend these findings by identifying impaired antiviral immunity to RV through limited production of IFN-β/IFN-λ and associated ISG induction in CF ACEs. This impaired antiviral status was restored by topical administration of IFN-β/IFN-λ, which are critical mediators of the host innate immune response against respiratory viruses.<sup>12–13</sup> These novel studies provide a mechanism to explain the previous observations with STAT1/NOS2 and underscore the potential therapeutic benefit of IFN therapy for RV infections in patients with CF.

We first identified differences in innate antiviral responses between CF and non-CF cells using three different well-characterised cell lines. Our data show that all cell lines have a similar phenotype with regard to impaired antiviral responses. We conclude that this is not an artefact of cell line immortalisation or transformation or specific to a particular cell line.<sup>35</sup> These results were confirmed in primary AECs from patients with CF and healthy controls. This is the first time impaired innate antiviral immunity has been identified in both nasal and





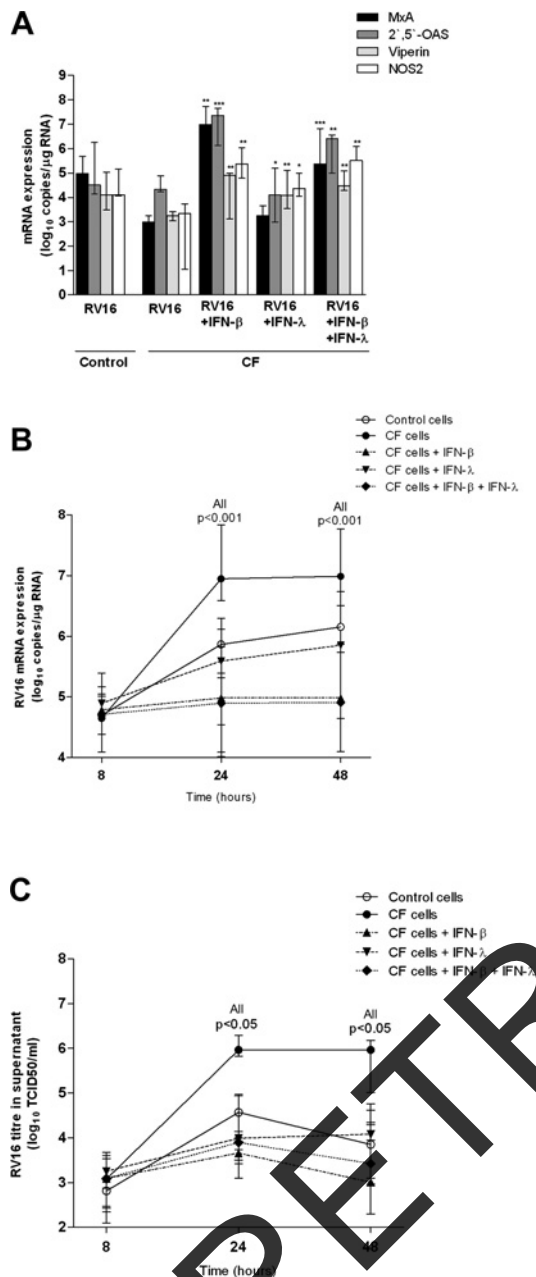
**Figure 3** Deficient induction of interferon-stimulated genes in CF bronchial epithelial cells after RV infection. (A) CF (UNCCF2T) and control (UNCCN2T) bronchial epithelial cells were infected with RV16 or treated with medium alone [M]. Myxovirus resistance A (MxA),

bronchial cells in CF. This is important, as many respiratory virus infections may start as upper airway infections and proceed to the lower airway. Experimental RV infections are often given in the nose and proceed to the lung causing lower airway inflammation, symptoms and reduction in lung function.<sup>36–38</sup> Our data support a hypothesis that impaired antiviral immunity in the nose may lead to greater virus dissemination and hence greater risk of lower airway infection.

To understand mechanisms behind the increased susceptibility of the CF airway epithelium towards RVs, we investigated epithelial IFN responses. We observed significant IFN- $\beta$ , IFN- $\lambda$ 1 and IFN- $\lambda$ 2/3 production after infection in both CF and non-CF, which is consistent with data from the literature reporting that IFN- $\beta$  and IFN- $\lambda$  but not IFN- $\gamma$  or IFN- $\alpha$  are produced by the airway epithelium in response to viruses.<sup>14</sup> IFN induction was profoundly impaired in CF cells at the mRNA expression and protein level. Impaired IFN production was a selective defect, as the proinflammatory cytokine response in CF ACEs was similar in CF and non-CF. The relevance of this defective IFN production was demonstrated by the inverse relationship between IFN production and virus replication: whereas primary AECs obtained from patients with CF produced low levels of IFN and were unable to control virus replication, healthy AECs produced high levels of IFN associated with low virus replication. The addition of exogenous IFN to CF cells restored control of virus replication, proving that IFN deficiency is the cause of impaired virus control in CF. Zheng *et al.*<sup>32, 33</sup> previously reported increased parainfluenza replication in CF AECs from lung explants. They identified defective IFN signalling with impaired induction of NOS2 and 2',5'-OAS as possible mechanisms, but did not study IFN- $\beta$ , and the IFN- $\lambda$  family was only very recently described at the time. Our findings of impaired IFN induction associated with deficient induction of ISGs including NOS2 and 2',5'-OAS suggest that their results<sup>32, 33</sup> were a consequence of defective IFN induction, although we cannot rule out the possibility that other mechanisms independently contribute to impaired antiviral control of the CF airway epithelium. Others have reported similar IFN responses upon RV infection in well-differentiated AECs from patients with CF and healthy controls.<sup>39</sup> Technical issues such as virus strains used, infection intensity and cell culture conditions might account for these apparently dissimilar results. Although we did not formally rule out increased virus binding or endocytosis in CF cells, our findings of similar viral loads at early time points in CF and non-CF cells and of defective IFN induction argue against this mechanism as a cause of increased susceptibility of the CF airway epithelium towards RVs. In addition, similar expression of RV receptors

[Continued]

2',5'-oligoadenylate synthetase (OAS), viperin and nitric oxide synthase (NOS)-2 gene expression levels were measured by RT-qPCR at 8h post-infection. Expression of all ISGs increased in both CF and control cells compared to medium control ([M]) (\*= $p < 0.05$ , \*\*= $p < 0.01$ ; \*\*\*= $p < 0.001$ ), but was significantly impaired in CF compared to control cells. Data are presented as median (IQR) of 3–4 independent experiments. (B) MxA gene expression was inversely related to virus replication. Linear regression  $R^2$  was 0.67,  $p = 0.001$ . (C) MxA gene expression was positively related to levels of IFN- $\beta$  protein in supernatant. Linear regression  $R^2$  was 0.73,  $p < 0.001$ . Associations remained significant after adjusting for disease group in multivariable regression analysis. White dots: control cells; black dots: CF cells.



**Figure 4** Exogenous interferon (IFN) restores the antiviral response to rhinovirus (RV) in cystic fibrosis (CF) bronchial epithelial cells. (A) Effect of exogenous IFN- $\beta$  and IFN- $\lambda$  (100 pg/ml) on myxovirus resistance A (MxA), 2',5'-oligoadenylate synthetase (OAS), viperin and nitric oxide synthase (NOS)-2 gene expression was tested at 8 h after RV16 infection by RT-qPCR in UNCCF2T cells. Both IFN- $\beta$  and IFN- $\lambda$  increased all IFN-stimulated genes (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ), but the effect was most pronounced for IFN- $\beta$ . (B) Virus replication in UNCCF2T and UNCN2T cells was measured by RT-qPCR at 8 h, 24 h and 48 h after RV16 infection in the presence or absence of exogenous IFN- $\beta$  and IFN- $\lambda$  (100 pg/ml). There was a reduction in virus RNA expression in CF cells treated with IFN- $\beta$  and/or IFN- $\lambda$  to the level of control cells ( $p < 0.001$  for both IFN- $\beta$  and IFN- $\lambda$ ). (C) RV16 release into the supernatants of infected cells was determined by calculating the 50% tissue culture infective dose (TCID<sub>50</sub>/ml) by titration assay on Ohio HeLa cells at 8 h, 24 h and 48 h after infection in the presence or absence of exogenous IFN- $\beta$  and IFN- $\lambda$  (100 pg/ml). There was a reduction in virus load in supernatants of CF cells treated with IFN- $\beta$  and/or IFN- $\lambda$  to the level of control cells ( $p < 0.05$  for both IFN- $\beta$  and IFN- $\lambda$ ). Data are presented as median (IQR) of 3–4 independent experiments.

**Table 2** Characteristics of patients with cystic fibrosis (CF) and healthy control subjects

	Gender	Age (years)	Atopy*	Steroid use†	FEV <sub>1</sub> (%)	PA colonisation‡	Genotype
<b>Nasal cells</b>							
<b>Controls</b>							
1	M	26.5	No	No			
2	F	29.9	No	No			
3	F	26.9	No	No			
4	F	29.5	No	No			
5	M	48.9	No	No			
6	F	27.1	No	No			
7	F	25.4	No	No			
8	M	15.1	No	No			
9	M	14.9	No	No			
10	M	6.2	No	No			
11	M	5.9	No	No			
12	M	3.9	No	No			
13	F	25.4	No	No			
14	M	27.9	No	No			
15	M	33.3	No	No			
16	M	25.1	No	No			
<b>CF patients</b>							
1	F	17.8	No	No	85	Yes	F508del/F508del
2	M	22.5	No	No	84	Yes	F508del/F508del
3	M	23.9	No	No	21	Yes	F508del/F508del
4	F	16.5	No	No	104	Yes	F508del/F508del
5	M	13.7	No	No	94	No	F508del/G126D
6	F	13.5	No	No	86	Yes	F508del/F508del
<b>Bronchial cells</b>							
<b>Controls</b>							
1	M	14.3	No	No			
2	M	4.4	No	No			
3	F	8.7	No	No			
4	F	16.1	No	No			
5	M	2.7	No	No			
6	F	10.5	No	No			
7	M	4.0	No	No			
8	F	12.2	No	No			
<b>CF patients</b>							
1	M	0.1	No	No	ND	No	F508del/F508del
2	F	14.2	No	No	85	Yes	F508del/3905insT
3	F	29.1	No	No	66	No	F508del/F508del
4	M	10.4	No	No	82	Yes	Q525X/ Q525X
5	M	27.7	No	No	88	Yes	F508del/G542X
6	F	15.9	No	No	73	No	F508del/F508del
7	M	0.3	No	No	ND	No	F508del/R553X

\*Atopy defined as a positive history of hay fever, eczema or asthma.

†Steroid use defined as any treatment with systemic, inhaled or nasal steroids within the past 3 months.

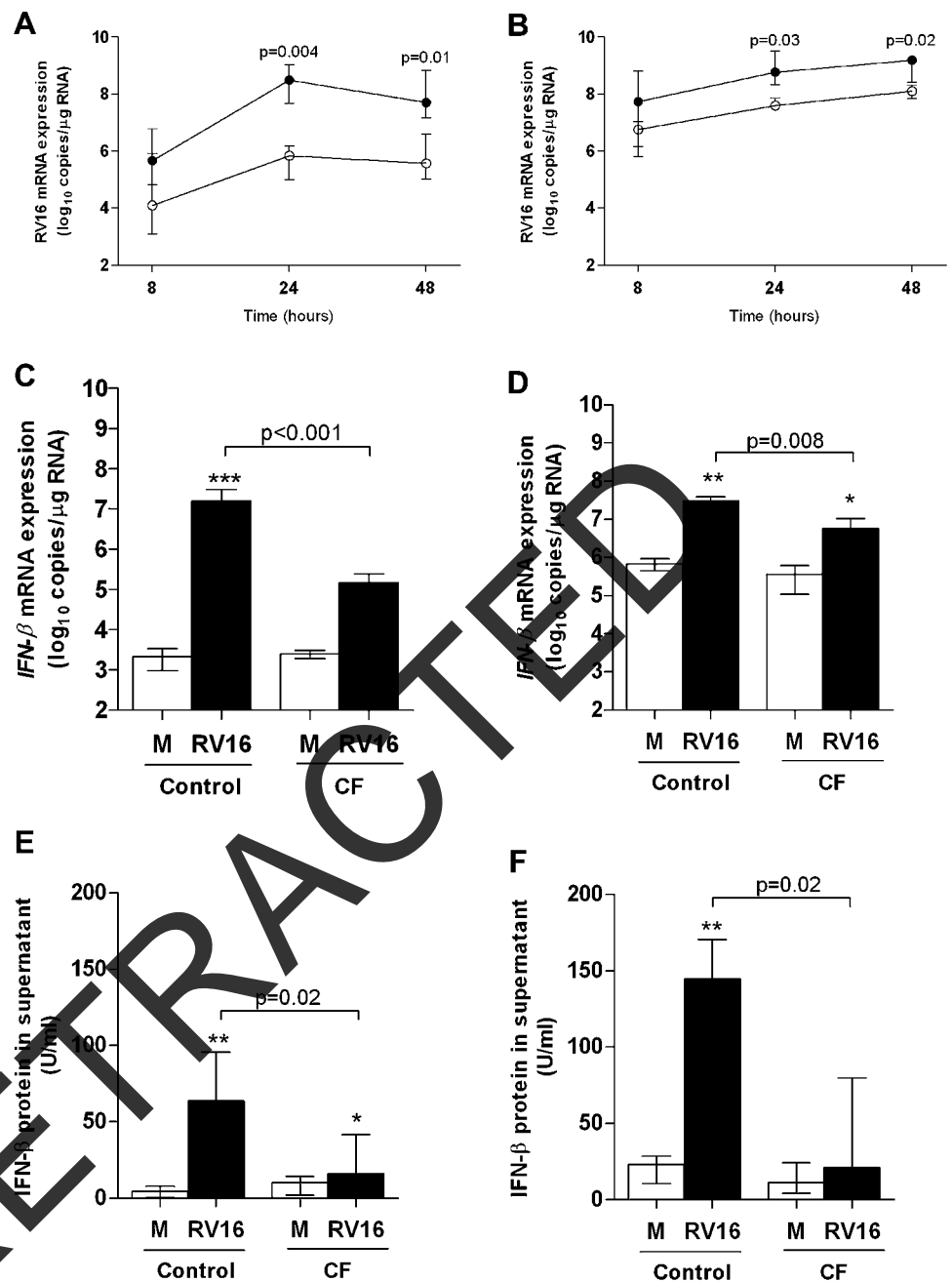
‡*Pseudomonas aeruginosa* (PA) colonisation defined as one or more PA-positive oropharyngeal cultures during the preceding 12 months; negative: no growth of PA in previous 12 months.

FEV<sub>1</sub>, forced expiratory volume in 1 s as percentage predicted; ND, not done.

(ICAM-1, sICAM-1 and VLDL) and viral binding/endocytosis in CF and control cells has recently been reported.<sup>39–41</sup>

Our findings may have direct therapeutic relevance as we corrected impaired virus control and defective ISG induction by adding exogenous IFN. IFN- $\beta$  had the most pronounced effect, suggesting it as the preferred candidate. Addition of IFN- $\beta$  restored levels of ISGs to control levels, which was not achieved by the addition of IFN- $\lambda$  in our study nor by the addition of IFN- $\gamma$  or a cytokine cocktail in the study by Zheng *et al*<sup>32</sup>. So far, there has been only one therapeutic trial using IFN

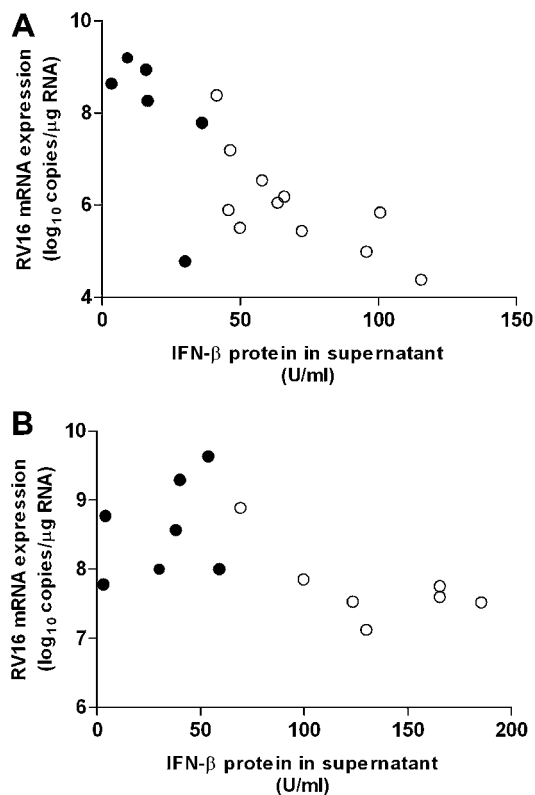
**Figure 5** Impaired virus control and interferon (IFN) production in primary cystic fibrosis (CF) airway epithelial cells. Virus load was measured by RT-qPCR 8 h, 24 h and 48 h after RV16 infection of (A) primary nasal epithelial cells and (B) bronchial epithelial cells obtained from patients with CF or healthy control donors. Virus replication increased over time and was significantly higher in CF cells (closed circles) than in control cells (open circles). RV16 induced IFN- $\beta$  mRNA expression at 8 h after infection in both (C) primary nasal and (D) bronchial CF and control cells (\* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001 vs medium control (M)). However, IFN- $\beta$  mRNA expression was significantly impaired in CF compared with control cells. Production of IFN- $\beta$  protein in (E) primary nasal and (F) bronchial epithelial cell supernatants was increased in both CF and non-CF cells at 48 h after RV16 infection (\* $p$ <0.05, \*\* $p$ <0.01 vs M). However, production of IFN- $\beta$  protein was significantly impaired in CF cells compared with non-CF cells. Data are presented as median (IQR) of 6 CF subjects and 16 healthy controls for nasal cells and of 7 CF subjects and 8 healthy controls for bronchial cells.



application in patients with CF. Moss *et al*<sup>42</sup> tested the effect of aerosolised IFN- $\gamma$ 1b in patients with CF with mild to moderate lung disease but did not find any beneficial effect. The lack of efficacy of this approach may be due to the fact that IFN- $\gamma$ 1b is a type II IFN which is mainly produced by natural killer cells and T lymphocytes and not by the bronchial epithelium.<sup>43</sup> Topical type I IFNs have successfully been used in the treatment of severe asthma, leading to improved lung function and reduced need for oral corticosteroids.<sup>44</sup> A similar approach may benefit patients with CF.

We were able to gain an insight into the mechanisms leading to deficient IFN induction in the CF airway epithelium. We and others have recently demonstrated a lack of exaggerated inflammatory responses and increased AEC necrosis upon RV infection in CF.<sup>21 41</sup> Here we show that impaired IFN induction

is not a consequence of virus-induced increased cell death as exogenous IFN decreased cytotoxicity in CF cells to the level of control cells, but rather that impaired IFN induction results in enhanced cell death. Further, we found that deficient IFN production was not due to altered virus recognition by CF ACEs as expression of dsRNA PRRs was similar in CF and control cells. Our data suggest that aberrant PRR signalling leads to deficient IFN production in CF. Indeed, Chattoraj *et al* recently demonstrated inhibited RV-stimulated Akt phosphorylation and decreased interferon regulatory factor-3 phosphorylation in CF but not in normal AECs after *Pseudomonas aeruginosa* infection.<sup>39</sup> Susceptibility to RV infection associated with deficient IFN production is not unique to CF and has been shown in asthma<sup>15 16</sup> and COPD.<sup>45</sup> Because CF, asthma and COPD are all chronic inflammatory airway diseases, we speculate that

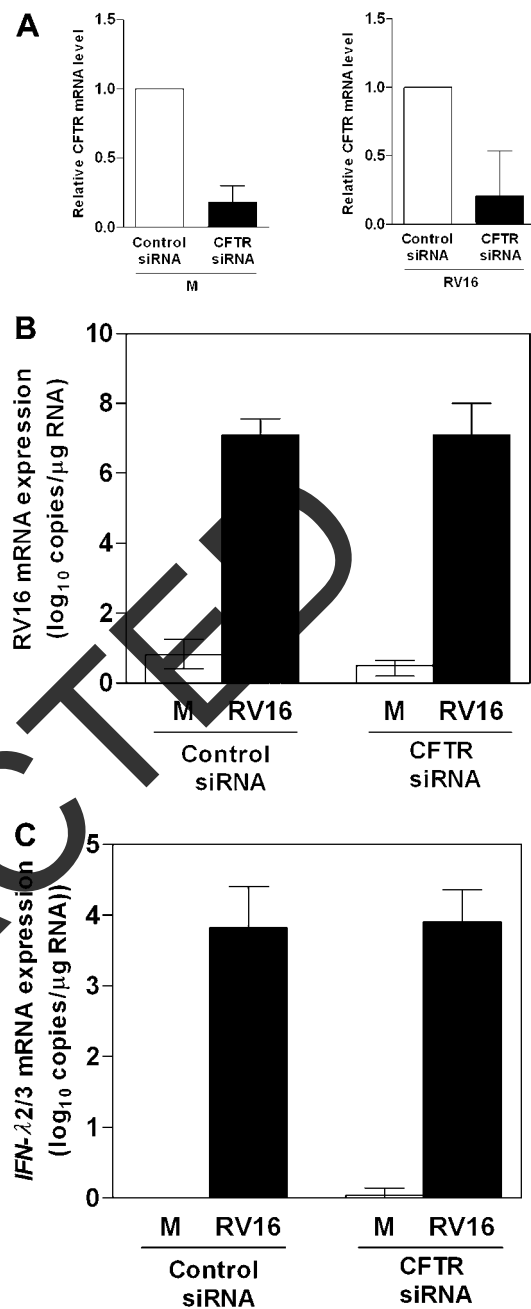


**Figure 6** Relationship between interferon (IFN) production and rhinovirus (RV) replication in cystic fibrosis (CF) primary airway epithelial cells. Levels of IFN- $\beta$  protein released 48 h after infection with RV16 by (A) primary nasal epithelial cells and (B) bronchial epithelial cells were inversely related to virus replication. Linear regression  $R^2$  was 0.59,  $p < 0.001$  for nasal cells and  $R^2$  was 0.29,  $p = 0.03$  for bronchial cells. Associations remained significant when adjusting for disease group in multivariable regression analysis. Data are presented as median (IQR) of 6–7 CF subjects and 7–11 healthy controls for bronchial cells. Open circles represent control cells and closed circles represent CF cells.

deficient antiviral responses are the consequence of a chronic proinflammatory state rather than directly linked to disease-specific characteristics such as CFTR dysfunction. Indeed, in our study, CFTR inhibition in healthy AECs did not lead to impaired IFN responses and higher virus replication. Recent evidence suggests that a chronic inflammatory milieu, such as that seen in the context of bacterial infection or atopy, suppresses IFN responses towards virus infections in the airways.<sup>39,46</sup> However, it might be that, in CF, both inflammatory milieu and loss of CFTR function lead to defective epithelial IFN production upon virus infection through, for example, increased and persistent oxidative stress.<sup>39</sup>

Our study has some limitations. We have used primary and immortalised ACEs grown as monolayers, and full differentiation of cells with addition of factors of the naturally occurring microenvironment may have been needed to better mimic virus effects on ACEs. Furthermore, the number of study subjects was relatively small, which did not allow us to examine the effects of genetic and environmental factors. Finally, we did not explore synergisms between viruses and bacteria which might be of relevance to antiviral responses of the CF airway epithelium.<sup>39</sup>

In conclusion, our findings demonstrate a deficient antiviral response to RV infection in CF AECs. Impaired induction of IFN- $\beta$ , IFN- $\lambda$  and ISGs results in increased virus replication. This defect can be restored in vitro by the addition of exogenous IFN



**Figure 7** Virus replication after CFTR inhibition in healthy primary airway epithelial cells. (A) CFTR levels in untreated (medium (M)) and RV16-infected primary nasal epithelial cells. (B) RV16 and (C) interferon (IFN)- $\lambda$ 2/3 mRNA expression were measured by RT-qPCR 24 h after infection in control and CFTR siRNA cells. Neither RV16 expression nor levels of IFN- $\lambda$ 2/3 mRNA differed between control and CFTR siRNA cells. Data are presented as median (IQR) of five healthy controls. CFTR, cystic fibrosis transmembrane conductance regulator; RV, rhinovirus; siRNA, small interfering RNA.

which limits virus replication to levels observed in normal cells. We therefore suggest that IFNs, particularly IFN- $\beta$ , might provide novel therapeutic avenues for the treatment of virus-induced CF exacerbations for which very few therapeutic options currently exist.

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**Competing interests** SLJ has received consulting/lecturing fees and/or research grants from AstraZeneca, Centocor, GlaxoSmithKline, Merck, Pfizer, Sanofi-Pasteur and Synaigen.

**Patient consent** Obtained.

**Ethics approval** The study protocol was approved by the ethics committee of the Canton of Bern, Switzerland.

**Contributors** Conception and design: MV, EK, MRE, NR. Acquisition of data: MV, EK, MPA, BSK, AM, NR. Analysis and interpretation: MV, EK, BSK, SLJ, MRE, NR. Drafting the manuscript for important intellectual content: MV, EK, MPA, BSK, AM, TG, SLJ, MRE, NR. Final approval of the manuscript: all authors.

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## Retraction

Marjolaine Vareille,<sup>1,2,3,4\*</sup> Elisabeth Kieninger,<sup>1,4\*</sup> Marco P. Alves,<sup>1,4</sup>  
Brigitte S. Kopf,<sup>1,4</sup> Alexander Möller,<sup>5</sup> Thomas Geiser,<sup>6</sup>  
Sebastian L. Johnston,<sup>7</sup> Michael R. Edwards,<sup>7</sup> and Nicolas Regamey<sup>1,4</sup>

<sup>1</sup>Department of Clinical Research, University of Bern, 3010 Bern, Switzerland

<sup>2</sup>Institute for Infectious Diseases, University of Bern, 3010 Bern, Switzerland

<sup>3</sup>Laboratoire d'Immunologie, EA 4233, Facultés de Médecine et Pharmacie, 63001 Clermont-Ferrand, France

<sup>4</sup>Division of Respiratory Medicine, Department of Paediatrics, University Children's Hospital of Bern, Inselspital, 3010 Bern, Switzerland

<sup>5</sup>Department of Respiratory Medicine, University Children's Hospital, 8091 Zürich, Switzerland

<sup>6</sup>Division of Respiratory Medicine, University Hospital of Bern, Inselspital, 3010 Bern, Switzerland

<sup>7</sup>Department of Respiratory Medicine, National Heart and Lung Institute, Wright Fleming Institute of Infection and Immunity & MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, Imperial College London, Norfolk Place, London W2 1PG, UK

**Vareille M, Kieninger E, Alves MP, et al. Impaired type I and type III interferon induction and rhinovirus control in human cystic fibrosis airway epithelial cells. *Thorax* 2012;67:517-25. This article has been retracted.**

In our article recently published in *Thorax*, we described a novel mechanism explaining the increased susceptibility of patients with cystic fibrosis (CF) to rhinovirus infections, namely defective interferon type I and III production by CF airway epithelial cells. In experiments performed after publication of the article we were unable to consistently replicate our findings of deficient interferon type I and III production by CF airway epithelial cells upon rhinovirus infection. In the light of these results, we carried out detailed investigations of the data reported in the above manuscript and regrettably found evidence of deliberate manipulation of experimental data by the first author Dr M. Vareille. This manipulation was accompanied in some instances by absence of original data files. The manipulation/original data absence involved data presented in most, if not all of the figures, thus we wish to fully retract the paper and apologise to the readers of *Thorax* and to the scientific community for the inconvenience this has caused.

We also checked data published by our group in manuscripts on which Dr Vareille was a co-author and found that data published in these manuscripts had not been manipulated. These two manuscripts, whose data and conclusions we stand by are:

Edwards MR, Regamey N, Vareille M, et al. Impaired innate interferon induction in severe therapy resistant atopic asthmatic children. *Mucosal Immunol* 2013;6:797–806. doi: 10.1038/mi.2012.118.

and

Kieninger E, Vareille M, Kopf BS, et al. Lack of an exaggerated inflammatory response on virus infection in cystic fibrosis. *Eur Respir J* 2012;39:297–304. doi: 10.1183/09031936.00054511.

Dr. Vareille has received a letter from the Secretary General of the University of Bern condemning her scientific misconduct as a severe offence against the rules of scientific integrity. Her current employers have also been informed.

All co-authors of the publication including Dr. Vareille concur with the retraction statement.

*Thorax* 2013;68:886. doi:10.1136/thoraxjnl-2011-200405ret