Online Supplement

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

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SUPPLEMENTAL METHODS

**Cell culture.** UNCCF2T/UNCN2T cells were grown in CnT-17 medium (CELLnTEC, Switzerland) supplemented with 1% Penicillin/Streptomycin and 10% fetal calf serum (FCS, Invitrogen US) in a humidified incubator (37°C, 5% CO₂). CFBE41o-/16HBE14o- cells were grown in Minimum Essential Medium and IB3-1/IB3-S9 cells in Dulbecco’s Modified Eagle Medium plus Glutamax, both additionally supplemented as above (InvivoGen, US).

**Virus culture.** The identities of each RV were confirmed by neutralisation using serotype specific antibodies. Virus stocks were negative for *Mycoplasma* infection.

**Primary human airway epithelial cells.** Nasal AECs were obtained by brushing the inferior surface of the middle turbinate of both nostrils with a 3-mm-cytology brush (Dent-o-Care, UK). Bronchial AECs were grown from brushings of the bronchial tree performed during clinically-indicated bronchoscopies or through the endotracheal tube in anaesthetised subjects undergoing elective surgery [E1]. Primary cultures were established by seeding freshly brushed cells into Bronchial Epithelial Growth Medium supplemented with Single Quots (Lonza, Switzerland), Primocin (100µg/ml; InvigoGen, US) and 10% FCS (Invitrogen, US). CF AECs were additionally treated with ceftazidime (100µg/ml; GlaxoSmithKline, Switzerland) and amphotericin B (2.5mg/l; Sigma, US) during first days of culture. Immunocytochemical staining for cytokeratin-13 and -19 (Abcam, UK) and occludin (Invitrogen, US) confirmed epithelial origin >95% of the cells obtained. At passage two, cells were seeded onto 12-well plates (Nunc, Rochester, US) until 80-90% confluency and placed into Bronchial Epithelial Basal Medium (Lonza, Switzerland) without any supplements for 24h prior to infection.
RT-qPCR. Cell lysates were obtained by adding 350µl of RLT-buffer (Qiagen, Switzerland). Total RNA was extracted (RNeasy Kit, Qiagen, Switzerland) and 2µg used for cDNA synthesis (Omniscript-RT-Kit, Qiagen, Switzerland). qPCR was carried out using specific primers (sense 300nM; antisense 900nM) and probes (175nM) for RV, IFN-λ1, IFN-λ2/3, IFN-β, 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 (Supplemental Table E1). Reactions consisted of 2µl of cDNA (cDNA for 18S was diluted 1:100) and 12.5µl QuantiTect-Probe PCR-MasterMix (Qiagen, Switzerland) and were performed on iCycler® (Biorad, US). Gene expression was normalized to 18S rRNA, which has been shown to be a stable housekeeping gene for studies involving human airway epithelial cells [E2], and expressed as copies per µg of total RNA using a standard curve generated by amplification of plasmid DNA bearing the cDNA of the gene of interest.

Table E1. Sequences of primers and probes

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<tr>
<th>Gene</th>
<th>Sequence of primers and probes</th>
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<tbody>
<tr>
<td>RV</td>
<td>Forward-5’-GTGAAGAGGCSCRTGTGCT-3’&lt;br&gt;Reverse-5’-GCTSCAGGTTAAGTAGCC-3’&lt;br&gt;Probe-5’-FAM-TGAGTCTCCGGCCCTGAATG-TAMRA-3’</td>
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<td>IFN-β</td>
<td>Forward-5’-CGCCGCATTGACCATCACA-3’&lt;br&gt;Reverse-5’-GACATTAGCCAGGGTTCTCA-3’&lt;br&gt;Probe-5’-FAMTCAGACAAGATTACATCGACTGCTGATAMRA-3’</td>
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<td>IFN-λ1</td>
<td>Forward-5’-GGACGCCTTGGAGAGTCACT-3’&lt;br&gt;Reverse-5’-AGAAGCCTCAGGTCCCAAATCTC-3’&lt;br&gt;Probe-5’-FAMAGGTCAGCTCTGCTTCCTCCCGATAMRA-3’</td>
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<td>IFN-λ2/3</td>
<td>Forward-5’-CTGCCCAATAGCCAGTCCA-3’&lt;br&gt;Reverse-5’-AGAAGCGACTCTCTTCTAAAGCATCCTT-3’&lt;br&gt;Probe-5’-FAMTCCTCACAGGAGCTGAGCCCTTATAMRA-3’</td>
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<td>IL-8</td>
<td>Forward-5’-TTGCCAGCTCTTCTCATT-3’&lt;br&gt;Reverse-5’-TATGCACCTGATCTAAGTTATCTCTATGCA-3’&lt;br&gt;Probe-5’-FAMCCTTGGCAGAA ACTGACACCATCATCTACATAMRA-3’</td>
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<td>MxA</td>
<td>Forward-5’-CAGCACCTGTGGCCTATCACC-3’&lt;br&gt;Reverse-5’-CATGACTGGATGAAAAGG-3’&lt;br&gt;Probe-5’-FAMAGGCAGCAGGGAGCTCAGTATAMRA-3’</td>
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<tr>
<td>Viperin</td>
<td>Forward-5’-CACAAAGAGGTTCTGGTTG-3’&lt;br&gt;Reverse-5’-AGCGCATATATTTTCATCAGGAATAAG-3’</td>
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**Probe-5'**-FAMCCTGAATCTAACCAGAAGATGAAAGACTCCTAMRA-3'

**2',5'-OAS**
- Forward-5'-CTGACFCTGACCTGGTTGTCT-3'
- Reverse-5'-CCCCGGCGATTAACTGAT-3'
- Probe-5'-FAMCCCTCAGTCTCCTACCACTTTTCATAMRA-3'

**NOS2**
- Forward-5'-GGTGGAAGCGGTAAACAAGG-3'
- Reverse-5'-TGCTTGGTGCGCGAAGATGA-3'
- Probe-5'-FAMAGAAACAAAGGACCTCAACTGAGCGGTAMRA-3'

**TLR3**
- Forward-5'-AAATTAAGAGTTTTCTCCAGGGTGTT-3'
- Reverse-5'-ATTCCGAATGCTTGTGTTTGC-3'
- Probe-5'-FAM-TTTGCGCTCTTTCTGAACATGTCCAGC-TAMRA-3'

**RIG-I**
- Forward-5'-CCAAGCAGAAGCGATTTTCAA-3'
- Reverse-5'-CACATGGGATCCCACTATG-3'
- Probe-5'-FAM-TTGAAAAAAGAGCAAATATTCTGTGCCCTGAC-TAMRA-3'

**MDA5**
- Forward-5'-GATTCCAGGACATGGAAGT-3'
- Reverse-5'-AGGCTGAGCTGAGGTCTG-3'
- Probe-5'-FAM-GGGATGCTCTTGACTGACATCTTTCT-TAMRA-3'

**CFTR**
- Forward-5'-AGCTGTCAAGCCGTGTTCTAGATA-3'
- Reverse-5'-ATGAGGATGCTGAGCGGACTTACC-3'
- Probe-5'-FAM-CACACGAAAATGACTGCAAGCCAGCT-TAMRA-3'

**18S**
- Forward-5'-CGCCGCTAGGGTGAAATCT-3'
- Reverse-5'-CATTCTGCAACAAATCTCTCG-3'
- Probe-5'-FAMACCGGCAGAAGGACGACCAGATAMRA-3'

**ELISA.** IFN-β and IL-8 proteins were quantified in supernatants from untreated and infected cell cultures using ELISA-kits for human IFN-β (Biosource International, US) and IL-8 (Amersham Biosciences, US) according to the manufacturer’s instructions. The detection limits were 2.5U/ml (IFN-β) and 3.5pg/ml (IL-8). To quantify IFN-λ1 in supernatants, we used a monoclonal anti-human IFN-λ1 as capture-antibody, a polyclonal anti-IFN-λ1 as secondary-antibody and biotin-conjugated donkey-anti-goat-IgG as third-antibody (R&D Systems, US), followed by streptavidin-conjugated horseradish-peroxidase (Biosource, US). Recombinant human IFN-λ1 (Peprotech, US) was used as standard. The sensitivity of the assay was 25pg/ml. This assay also detects IFN-λ2/3 due to 25% cross-reactivity [E3].

**Biological effect of IFN-β and IFN-λ.** Cells were seeded and treated for 24h before and for up to 48 hours after RV infection with/without different doses of IFN-β and/or IFN-λ1
Cytotoxicity. Cytotoxicity was assessed by measuring LDH activity in culture supernatants (Cytotoxicity Detection Kit; Roche, Switzerland).

Transfection and RNA interference. Healthy primary nasal AECs were cultured until 80-90% confluency and transfected with 100pmol of scrambled control or three different CFTR siRNAs by using lipofectamin 2000 (Invitrogen, US) according to manufacturer’s instructions. Following sequences of functional-siRNAs were used: siCFTR.1 (5’-CGUGUGUCUGUAAACUGAUGGCUAA-3’), siCFTR.2 (5’-CCCUUCUGUUGAUU-CUGCUGACAAU-3’), siCFTR.3 (5’-GGCAUAGGCUUAUGCC UUCUCUUUA-3’). Control-siRNA for the exclusion of non-target effects was 5’-UACCGUCUCCACUUAGUCGdTdT-3’ (E4).

Statistics. Descriptive statistics and analyses were performed using Stata™ (STATA Corporation, College Station, US). As most of the data were not normally distributed, we present them as median (interquartile range [IQR]). P-values <0.05 were considered statistically significant.
SUPPLEMENTAL REFERENCES


SUPPLEMENTAL FIGURE LEGENDS

Figure E1. Exogenous IFN decreases RV-induced cytotoxicity in CF bronchial epithelial cells (UNCN2T/UNCCF2T cell line). Cytotoxicity (%), assessed by measuring LDH activity in supernatants, was determined after exposure to RV16 in the presence or absence of exogenous IFN-β (100 pg/ml) and IFN-λ (100 pg/ml) at different timepoints (8h, 24h, 48h) and compared to untreated cells. Cytotoxicity was significantly increased in CF cells compared to non-CF cells (p=0.02). There was a reduction in cytotoxicity in CF cells treated with IFN-β and/or IFN-λ to the level of control cells (p=0.004 for both IFN-β and IFN-λ). Data are presented as median (IQR) of 3-4 independent experiments.

Figure E2. Similar expression of double-stranded RNA recognition receptors in CF and non-CF primary airway epithelial cells. Basal expression of MDA5 (A), TLR3 (B) and RIG-I (C) were measured by RT-qPCR in primary bronchial epithelial cells obtained from CF patients or healthy control donors. Expression of all receptors was similar in CF and non-CF cells. Data are presented as median (IQR) of 4 CF subjects and 4 healthy controls.