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 (37ºC, 5% CO_2). 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

Gene	Sequence of primers and probes
RV	Forward-5'-GTGAAGAGCCSCRTGTGCT-3' Reverse-5'-GCTSCAGGGTTAAGGTTAGCC-3' Probe-5'-FAM-TGAGTCCTCCGGCCCCTGAATG-TAMRA-3'
IFN-β	Forward-5'-CGCCGCATTGACCATCTA-3' Reverse-5'-GACATTAGCCAGGAGGTTCTCA-3' Probe-5'-FAMTCAGACAAGATTCATCTAGCACTGGCTGGATAMRA-3'
IFN-λ1	Forward-5'-GGACGCCTTGGAAGAGTCACT-3' Reverse-5'-AGAAGCCTCAGGTCCCAATTC-3' Probe-5'-FAMAGTTGCAGCTCTCCTGTCTTCCCCGTAMRA-3'
IFN-λ2/3	Forward-5'-CTGCCACATAGCCCAGTTCA-3' Reverse-5'-AGAAGCGACTCTTCTAAGGCATCTT-3' Probe-5'-FAMTCTCCACAGGAGCTGCAGGCCTTTATAMRA-3'
IL-8	Forward-5'-TTGGCAGCCTTCCTCATTTC-3' Reverse-5'-TATGCACTGACATCTAAGTTCTTTAGCA-3' Probe-5'-FAMCCTTGGCAAAACTGCACCTTCACACATAMRA-3'
MxA	Forward-5'-CAGCACCTGATGGCCTATCAC-3' Reverse-5'-CATGAACTGGATGATCAAAGG-3' Probe-5'-FAMAGGCCAGCAAGCGCATCTCCAGTAMRA-3'
Viperin	Forward-5'-CACAAAGAAGTGTCCTGCTTGGT-3' Reverse-5'-AAGCGCATATATTTCATCCAGAATAAG-3'

	Probe-5'-FAMCCTGAATCTAACCAGAAGATGAAAGACTCCTAMRA-3'
2',5'-OAS	Forward-5'-CTGACFCTGACCTGGTTGTCT-3' Reverse-5'-CCCCGGCGATTTAACTGAT-3' Probe-5'-FAMCCTCAGTCCTCTCACCACTTTTCATAMRA-3'
NOS2	Forward-5'-GGTGGAAGCGGTAACAAAGG-3' Reverse-5'-TGCTTGGTGGCGAAGATGA-3' Probe-5'-FAMAGAAACAACAGGAACCTACCAACTGACGGGTAMRA-3'
TLR3	Forward-5'-AAATTAAAGAGTTTTCTCCAGGGTGTT-3' Reverse-5'-ATTCCGAATGCTTGTGTTTGC-3' Probe-5'-FAM-TTTGGCCTCTTTCTGAACAATGTCCAGC-TAMRA-3'
RIG-I	Forward-5'-CCAAGCCAAAGCAGTTTTCAA-3' Reverse-5'-CACATGGATTCCCCAGTCATG-3' Probe-5'-FAM-TTGAAAAAAGAGCAAAGATATTCTGTGCCCGAC-TAMRA-3'
MDA5	Forward-5'-GATTCAGGCACCATGGGAAGT-3' Reverse-5'-AGGCCTGAGCTGGAGTTCTG-3' Probe-5'-FAM-GGGATGCTCTTGCTGCCACATTCTCTT-TAMRA-3'
CFTR	Forward-5'-AGCTGTCAAGCCGTGTTCTAGATA-3' Reverse-5'-ATGAGGAGTGCCACTTGCAAA-3' Probe-5'-FAM-CACACGAAATGTGCCAATGCAAGTCCTT-TAMRA-3'
18S	Forward-5'-CGCCGCTAGGGTGAAATTCT-3' Reverse-5'-CATTCTTGGCAAATGCTTTCG-3' Probe-5'-FAMACCGGCGCAAGACGGACCAGATAMRA-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-λ_f 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

(Peprotech, US).

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'-UACCGUCUCCACUUGAUCGdTdT-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

E1. McNamara PS, Kicic A, Sutanto EN, et al. Comparison of techniques for obtaining lower airway epithelial cells from children. *Eur Respir J* 2008;32(3):763-8.

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E3. Contoli M, Message SD, Laza-Stanca V, et al. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med* 2006;12(9):1023-6.

E4. Luciani A, Villella VR, Esposito S, et al. Defective CFTR induces aggresome formation and lung inflammation in cystic fibrosis through ROS-mediated autophagy inhibition. *Nature cell biology*;12(9):863-75.

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.