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Original Article

Interferon response of the cystic fibrosis bronchial epithelium to major and minor group rhinovirus infection

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Abstract

Rhinoviruses (RVs) are associated with exacerbations of cystic fibrosis (CF), asthma and COPD. There is growing evidence suggesting the involvement of the interferon (IFN) pathway in RV-associated morbidity in asthma and COPD. The mechanisms of RV-triggered exacerbations in CF are poorly understood. In a pilot study, we assessed the antiviral response of CF and healthy bronchial epithelial cells (BECs) to RV infection, we measured the levels of IFNs, pattern recognition receptors (PRRs) and IFN-stimulated genes (ISGs) upon infection with major and minor group RVs and poly(IC) stimulation. Major group RV infection of CF BECs resulted in a trend towards a diminished IFN response at the level of IFNs, PRRs and ISGs in comparison to healthy BECs. Contrary to major group RV, the IFN pathway induction upon minor group RV infection was significantly increased at the level of IFNs and PRRs in CF BECs compared to healthy BECs.

Keywords: Cystic fibrosis; Rhinovirus; Interferon; Bronchial epithelial cells

1. Introduction

Rhinoviruses (RVs) are small positive-sense ssRNA viruses belonging to the *Picornaviridae* family. RV serotypes are classified as major or minor group depending on the surface receptor used to infect target cells. More than 90% of RV serotyped strains belong to the major group and use as receptor the intercellular adhesion molecule 1 (ICAM-1), while the minor group RV strains bind to low-density lipoprotein receptor (LDLR) on target cells [1,2]. As the causative agents of the common cold and acute respiratory tract infections in children, RVs are one of the major causes of morbidity and mortality [3]. In addition, RVs are the predominant agents associated with pulmonary exacerbations of CF lung disease as they are detected in up to 40% of all virus-associated CF exacerbations [4–6]. The mechanisms of acute virus-mediated exacerbations in CF are so far poorly understood.

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We and others have reported recently that primary CF bronchial epithelial cells (BECs) have an increased susceptibility to respiratory virus infections such as RVs and parainfluenza virus [7–9]. We have confirmed these findings ex vivo in bronchoalveolar lavage (BAL) samples collected from RV-infected CF children, in which RV load was elevated in comparison to control patients [10].

RVs are also responsible for more than 50% of virus-induced asthma exacerbations [11]. Defective interferon (IFN) type I (IFN-β) and type III (IFN-λs) production of the bronchial airway epithelium upon RV infection has been identified as a contributing mechanism behind the impaired RV control in asthmatic adults and children [12–16]. The work of two groups studying mechanisms of viral control and IFN induction in BECs and macrophages collected from patients with chronic obstructive pulmonary disease (COPD) led to controversial results. After growing BECs at air–liquid interface (ALI), Schneider et al. showed an increase in IFN production upon RV infection. Despite that, cells showed an impaired viral control and an increased pro-inflammatory phenotype [17]. On the other hand, Mallia et al. found lower IFNs responses upon infection with RV by BAL macrophages from COPD patients compared to control subjects [18].

Since the IFN pathway is involved in the defective control of RV in infected BECs from asthmatic and COPD patients, we decided to evaluate the IFN response of CF BECs after challenging with RVs from the major and minor group (RV16 and RV1B, respectively). Also, we aimed to assess the baseline expression and induction of pattern recognition receptors (PRRs) engaged in the sensing of RV including toll-like receptor 3 (TLR3), melanoma differentiation-associated protein 5 (MDA5), and retinoic acid inducible gene I (RIG-I) [19]. Levels of IFN-stimulated genes (ISGs) such as dsRNA protein kinase R (PKR), 2′-5′-oligoadenylate synthetase 1 (OAS1), MxA (Myxovirus resistance gene A), and viperin (virus inhibitory protein, endoplasmic reticulum-associated, IFN inducible) were also measured. Finally, the inflammatory response mediated by RV infection of CF BECs has also been quantified through the measurement of CXCL8/IL-8, IL-6 and CXCL10/IP-10 cytokines release.

2. Material and methods

2.1. Study subjects

For the establishment of primary BEC cultures, we recruited healthy and CF volunteers at the University Hospitals of Bern and Zürich. The clinical characteristics of the participants used in this study have been presented elsewhere [9] and are reproduced here for clarity ease of reference (Table 1). The exclusion criteria were bleeding tendency, therapy with anticoagulants and/or immunosuppressive agents. For the control group steroid use within the past three months and atopy were additional exclusion criteria. The study was approved by the Ethics committees of the Cantons of Bern and Zurich, Switzerland and informed consent was obtained from all study participants and/or caregivers.

2.2. Isolation of primary CF and control bronchial epithelial cells

BECs from 11 CF and 12 control subjects were grown from bronchial brushings performed with a 3 mm brush (ConMed, USA) as described [9].

2.3. Cell culture

Primary submerged cultures of BECs were obtained by seeding freshly brushed cells in Bronchial Epithelial Growth Medium (BEGM, Lonza, Switzerland), supplemented with Single Quots (Lonza, Switzerland) as described previously [9].

2.4. Rhinovirus infection

RV16 and RV1B viruses were amplified and titrated with Ohio HeLa cells (ECCAC, UK). BECs were infected with RVs for 1 h at a multiplicity of infection of 4 and washed three times with PBS (Life Technologies, USA). Fresh medium was added and plates were further incubated at 37 °C for 24 h until harvesting. Cells were treated in parallel with infection media (IM) and polyinosinic–polycytidylic acid (poly(IC)) at a concentration of 1 μg/ml (Invivogen, USA). Since the peak of RV replication is observed at ca. 24 h post-infection [20,21], total RNA and supernatants were harvested at 24 h post-infection for further analysis.

2.5. Isolation of total RNA and RT-PCR

Total RNA purification was done by using the Nucleospin RNA II kit (Macherey-Nagel, Switzerland). Synthesis of cDNA was performed with Omniscript RT Kit (Qiagen, USA) following the manufacturer's protocol. RT-PCR measurements were done with the Taqman Fast universal PCR master mix and the Fast SYBR Green master mix on a 7500 Fast Real Time PCR System (all from Applied Biosystems, USA). The sequences of primers and probes are depicted in Table 2. To analyze the mRNA expression levels of IFNs, PRRs and ISGs, the ΔΔCt method was used [22]. The mRNA expressions levels were normalized to 18S rRNA.

2.6. ELISA

Protein levels of CXCL8/IL-8, IL-6 and CXCL10/IP-10 were measured by using the DuoSet ELISA Development kit (R&D, USA) with the following detection limits: CXCL8/IL-8 15 pg/ml, IL-6 3 pg/ml, and CXCL10/IP-10 2 pg/ml.

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., USA). The data were analyzed for normal distribution by using the Kolmogorov–Smirnov test. If normally distributed, paired data were analyzed with one-way ANOVA and the Tukey post hoc test. Paired data non-normally distributed were analyzed with the Friedman and the Dunn's