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Letter to the Editor

Synergistic suppression of Poly(I:C)-induced CCL3 by a corticosteroid and a long acting β2 agonist in nasal epithelial cells

Dear Editor

Chronic rhinosinusitis (CRS), bronchial asthma and COPD are chronic inflammatory diseases of the respiratory tract, and the major causes of acute exacerbations of these airway diseases are viral infections.[1,2] Viral infection induces the expression of a variety of proinflammatory molecules in the airway epithelium, which promotes inflammatory cell recruitment and activation.[3] Chronic inflammatory diseases of the upper and lower respiratory tracts often coexist in the same patients and suggest the 'united airways' concept.[1] Appropriate anti-inflammatory treatment of both the upper and lower airways is necessary to control them.[1,2]

Combinations of an inhaled corticosteroid (ICS) and long acting β2 agonist (LABA) are used to treat asthma and COPD, and reduce their acute exacerbations. Actually, combinations of an ICS and LABA synergistically suppress proinflammatory molecules produced by human rhinovirus-infected or polyinosinic-polycytidylic acid (Poly [I:C])-stimulated bronchial epithelial cells[4,5]; however, the influence of these drugs on nasal epithelial cells has not been examined.

CCL3, also called macrophage inflammatory protein-1α (MIP-1α), is a proinflammatory molecule produced in the airway by viral infections.[6] Bronchial epithelial cells produce CCL3 as a result of viral infections, and blockade of the CCL3 proinflammatory signaling cascade significantly reduces virus-induced lung inflammation.[6,7] Poly(I:C) mimics the effects of dsRNA intermediates produced in virus-infected cells. We first investigated mRNA expression and protein production of CCL3 in HNEpC after Poly(I:C) stimulation (Supplementary Methods). HNEpC were stimulated with Poly(I:C) (1 μg/mL) for 24 h, and we observed marked increases in mRNA after exposure to Poly(I:C) (Fig. 1a). We also found that Poly(I:C) increased the secreted protein levels of CCL3 ($P < 0.01$) (Fig. 1b).

Since normal human bronchial epithelial cells (NHBE) are known to have functional ADRB2,[8] we next examined whether functional ADRB2 was expressed on HNEpC. We observed the strongest mRNA expression in lung tissue in various human tissues (Supplementary Fig. 1) and found that *ADRB2* mRNA was as highly expressed in HNEpC as in NHBE (Fig. 1c). It is well known that ADRB2 stimulation results in an increase of intracellular cAMP.[9] Thus, we next investigated whether ADRB2 on HNEpC was involved in an increase in cAMP. We stimulated HNEpC with the ADRB2-specific agonist salmeterol or formoterol for 10 min and confirmed increased intracellular cAMP levels (Fig. 1d and e). Pretreatment with the ADRB2-specific antagonist ICI118,551 (5 μM) inhibited the increase of intracellular cAMP (Fig. 1d and e).

We further examined the suppressive effects of salmeterol and formoterol on the Poly(I:C)-induced *CCL3* mRNA expression and CCL3 production levels in HNEpC. Both salmeterol and formoterol suppressed the *CCL3* mRNA expression (Supplementary Fig. 2a and b) and levels of CCL3 protein production (Supplementary Fig. 2c and d). The ADRB2-specific antagonist ICI118,551 (5 μM) inhibited those suppressive effects of both agonists at the mRNA level (Supplementary Fig. 2a and b). The antagonist significantly inhibited the suppressive effect of formoterol on protein expression levels in all of the examined cell lines ($P < 0.01$) (Supplementary Fig. 2d). However, the antagonist significantly inhibited the suppressive effect of salmeterol in only three of the four examined cell lines (Supplementary Fig. 2c).

We next assessed the effects of dexamethasone and the LABAs salmeterol and formoterol on the induction of CCL3 by Poly(I:C). The *CCL3* mRNA expression was decreased by dexamethasone and/or a LABA (Fig. 2a and b), and synergistic suppression of *CCL3* by dexamethasone and salmeterol (Fig. 2a) or formoterol (Fig. 2b) was observed. Similar results were observed in all of the examined cell lines ($N = 4$). We then assessed the protein production levels of CCL3 by ELISA. We found significant differences between responses in both the salmeterol ($P < 0.05$) and the formoterol ($P < 0.05$) groups by the Kruskal–Wallis test (Fig. 2c and d). Post hoc analyses confirmed significant suppression of protein levels with concurrent exposure of the cells to dexamethasone and salmeterol ($P < 0.05$) (Fig. 2c) or formoterol ($P < 0.05$) (Fig. 2d). Similar results were observed in all of the examined cell lines ($N = 4$).

Airway epithelial cells are the first to be impacted by inhaled environmental factors and function in host defense and airway inflammation.[10] These cells act as an immunoregulator by producing various inflammatory mediators, and some of these mediators induced by respiratory viral infections are considered to contribute to exacerbations of chronic airway inflammatory diseases.[3,10] CCL3 is increased in both the upper and the lower respiratory secretions during viral infections, and bronchial epithelial cells produce CCL3 in the lower respiratory tract.[6] In this study, we found a marked increase in mRNA and secreted protein levels of CCL3 in Poly(I:C) stimulated HNEpC. Thus, nasal epithelial cells might be a source of CCL3 during viral infections of the upper respiratory tract.

Combinations of an ICS and LABA are widely used in anti-inflammatory therapy and reduce acute exacerbations of asthma and COPD. The combination therapy is more effective than ICS monotherapy, and the airway epithelium is a major target for the actions of inhaled medications.[10] ICS and LABA combinations synergistically suppress proinflammatory molecules produced by human rhinovirus-infected or Poly(I:C)-stimulated bronchial epithelial cells.[4,5,10] In this study, we observed that HNEpC expressed as

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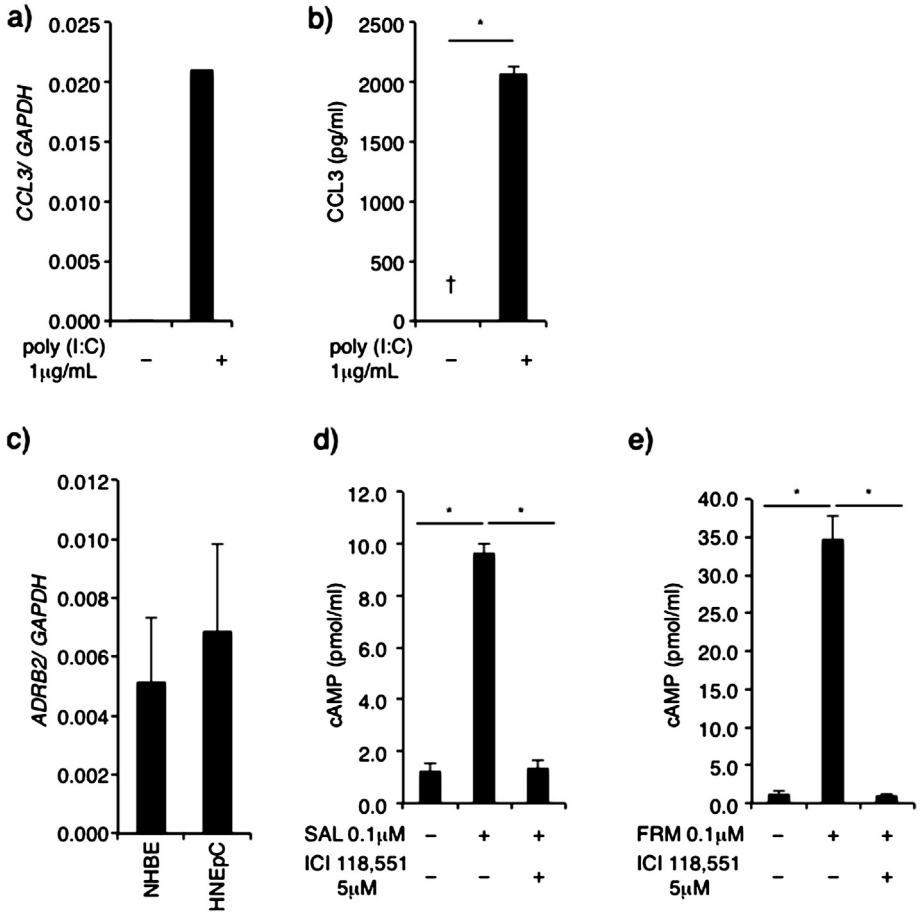

Fig. 1. CCL3 production by Poly(I:C) stimulated HNEpC and the presence of functional ADRB2 on HNEpC. a, b) HNEpC were stimulated with Poly(I:C) (1 μg/mL) for 24 h a) The *CCL3* mRNA expression level was measured by quantitative RT-PCR. Data are shown as the mean of triplicate samples and are representative of four separate experiments. b) The concentration of CCL3 protein in the supernatant was measured using ELISA. Data are shown as the mean ± SD of triplicate samples and are representative of four separate experiments. A dagger (†) indicates that the concentration was below the detection threshold (<7.81 pg/mL). c) The *ADRB2* mRNA expression levels of unstimulated resting NHBE and HNEpC. Data are shown as the mean ± SD of NHBE (N = 16) and HNEpC (N = 12). d, e) HNEpC were treated with ICI 118,551 (5 μM), and 30 min later, salmeterol (SAL) (0.1 μM) or formoterol (FRM) (0.1 μM) was added. Intracellular cAMP was measured by ELISA 10 min after stimulation with salmeterol d) or formoterol e). Data are shown as the mean ± SD of quadruplicate samples and are representative of three separate experiments. $^{*}P < 0.01$ by Welch's t-test.

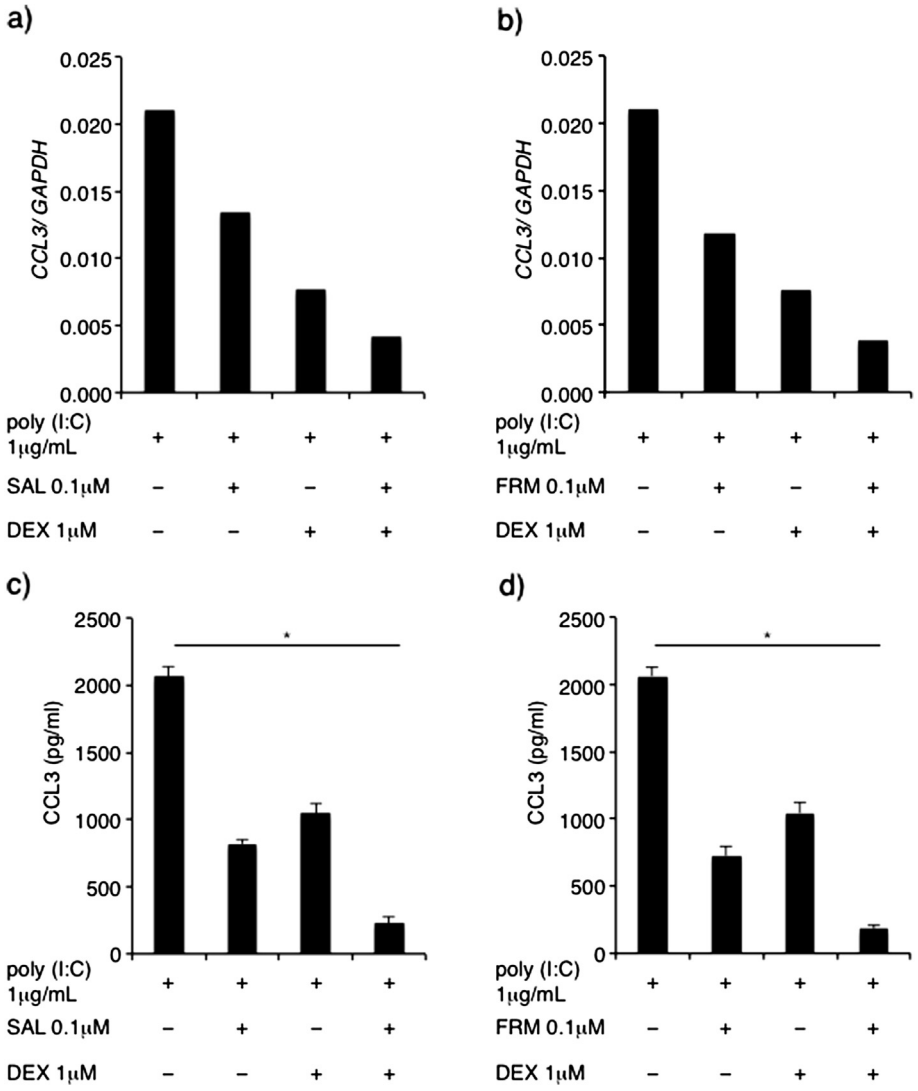

Fig. 2. Suppressive effects of long-acting beta agonists and dexamethasone on CCL3 production by Poly(I:C) stimulated HNEpC. HNEpC were treated with salmeterol (0.1 μM), formoterol (0.1 μM) and/or dexamethasone (1 μM), and the cells were stimulated with Poly(I:C) (1 μg/mL) for 24 h. The *CCL3* mRNA expression levels of cells treated with salmeterol a), formoterol b) and/or dexamethasone were measured by real-time RT-PCR. Data are shown as the mean of triplicate samples and are representative of four separate experiments. The concentrations of CCL3 protein in the supernatant treated with salmeterol c), formoterol d) and/or dexamethasone were measured using ELISA. Data are shown as the mean ± SD of triplicate samples and are representative of four separate experiments. $^{*}P < 0.05$ compared with Poly(I:C) stimulation by Scheffe's test.

much *ADRB2* mRNA as NHBE, and the intracellular cAMP level was increased in HNEpC by salmeterol or formoterol stimulation. We also found that the combination of dexamethasone and a LABA had a synergistic suppressive effect on CCL3 induction in HNEpC.

Corticosteroid nasal sprays are commonly used to treat allergic rhinitis; however, β2 agonists are not used as the principal medication option for rhinitis. Acute exacerbation of chronic airway inflammation is mostly started by upper airway viral infections.[1,2] Although further studies are needed, exhalation of the therapeutic agents through the nose might help to reduce CCL3 induction and lessen the burden of exacerbations in patients receiving combination therapy.

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Conflict of interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.alit.2014.10.006.

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