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Thymic stromal lymphopoietin and apocynin alter the expression of airway remodeling factors in human rhinovirus-infected cells

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ABSTRACT

Airway remodeling is a characteristic of bronchial asthma. The process involves the expression of many genes, such as transforming growth factor-beta (TGF-β), tissue inhibitors of metalloproteinases (TIMP-1), MMP and arginase. Human rhinovirus (HRV) is known to cause asthma exacerbations, and viral infections might be involved in the development of airway remodeling. Therefore, the aim of this study was to determine the influence of HRV on the genes involved in airway remodeling and to examine the impact of thymic stromal lymphopoietin (TSLP) and contribution of oxidative stress on airway remodeling in the context of HRV infection.

Peripheral blood mononuclear cells, isolated from blood collected from 10 healthy volunteers, and human lung fibroblasts were infected with HRV-16. The cells were treated with apocynin or TSLP 48 h after infection. The expression of TGF-β1, TIMP-1 and arginase I mRNA and protein were determined by real-time PCR, immunoblotting and ELISA, respectively.

Rhinovirus infection significantly increased the expression of TGF-β1 and arginase I, on the mRNA and protein levels. This effect was inhibited by apocynin, though only on the mRNA level. TIMP-1 expression was not influenced by HRV; however, apocynin caused a significant increase of TIMP-1 mRNA expression. TSLP increased the expression of TGF-β1 and arginase I mRNA in fibroblasts, but not in PBMC.

1. Introduction

Airway inflammation and remodeling are believed to interact with each other, resulting in the occurrence and progress of asthma (Grainge et al., 2011). Airway remodeling, the most typical pathological feature of asthma, is defined by several structural changes, including greater deposition of extracellular matrix proteins (ECMs), goblet cell metaplasia, angiogenesis and increased airway smooth muscle mass, which are involved in persistent airflow limitation and lower baseline lung function (Chiappara et al., 2001). Exacerbations, some of which are induced by HRV, can occur in asthma, and these are important enhancers of morbidity. Respiratory viral infections can have severe adverse outcomes in patients with established asthma and are associated with nearly 80% of asthma exacerbation episodes (Heymann et al., 2004; Tsukagoshi et al., 2013; Wark et al., 2002). Despite extensive clinical evidence linking HRV infection to illness of the lower airway, the precise role of HRV as a lower airway pathogen is still unclear, mainly because HRV is frequently recovered in asymptomatic individuals of those with very mild illness (Kim and Gern, 2012).

Recently, several independent genome-wide association studies have demonstrated that thymic stromal lymphopoietin (TSLP) acts as a susceptibility locus for asthma (Hirota et al., 2011; Torgerson et al., 2011; Wu et al., 2013). In airways, TSLP is produced by epithelial cells, mast cells, airway smooth muscle cells (ASMC) and fibroblasts, and is significantly elevated in asthma and allergic diseases (Brandelius et al., 2011; Kato et al., 2017; Kaur et al., 2012). TSLP induces the activity and expression of α-SMA and collagen I in fibroblasts, suggesting that TSLP plays a pivotal role in asthmatic airway remodeling, and fibroblasts is significantly elevated in asthma and allergic diseases (Mitchell and O'Byrne, 2017; Verstraete et al., 2017). TSLP induces activity and the expression of α-SMA and collagen I in fibroblasts, suggesting that TSLP plays a pivotal way in asthmatic airway remodeling (Watson and Gauvreau, 2014). Chemicals, viruses and allergens are implicated as stimuli for inducing TSLP production in inflamed tissue (Fontenot et al., 2009; Segawa and Hirasawa, 2014; Yi et al., 2017). TSLP has been shown to induce migration in DCs, suggesting a physiological function of TSLP that could potentially promote inflammation (Fernandez et al., 2011; Redhu et al., 2013; Redhu and Gounni, 2012). Previously Li et al. demonstrated that TSLP expression was increased in the airway epithelium of asthmatics and TSLP signaling was crucial for the

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generation of an inflammatory reaction in asthma (Li et al., 2010).

A body of evidence highlights the involvement of NADPH oxidase-dependent redox signaling in the profibrotic responses mediated by TGF-β (Chan et al., 2009; Jiang et al., 2014). Oxidative stress is known to augment airway remodeling by stimulating the production of transforming growth factor-β1 (TGF-β1), fibronectin and vascular endothelial growth factor (VEGF) in lung fibrosis (Bellocq et al., 1999; Qi et al., 2009; Yoon et al., 2016). It has also been demonstrated that apocynin, an inhibitor of NADPH oxidase activation, reduces reactive oxygen species concentrations in healthy subjects, as well as asthmatic and COPD patients (Stefanska et al., 2012a; Stefanska et al., 2012b; Stefanska et al., 2010).

Our previous studies demonstrated that apocynin – inhibitor of NADPH oxidase activation, reduced reactive oxygen species concentrations in healthy subjects, asthmatic and COPD patients (Stefanska et al., 2012a; Stefanska et al., 2012b; Stefanska et al., 2010).

At present, the predominant therapeutic option for patients with asthma is the use of anti-inflammatories. Despite intensive study, airway remodeling remains not fully understood, and is therefore impossible to prevent. Previous studies have shown that airway remodeling results in the progressive loss of lung function (Hartley et al., 2005; Pascual and Peters, 2005). Therefore, the aim of this study was to identify the role of TSLP and apocynin, an NADPH-oxidase inhibitor, on rhinovirus-induced airway remodeling and the genes involved in this process.

2. Results

2.1. Apocynin inhibits HRV-induced TGF-β1 expression in fibroblasts and PBMC

Real-time PCR analysis revealed that RV-16 infection significantly increased TGF-β1 mRNA expression levels in normal human lung fibroblasts (RQ = 7.02) and PBMCs (RQ = 5.86) ($p < 0.05$; Fig. 1A and B). Although the addition of apocynin suppressed this effect in both cell types, a significant difference was found between fibroblasts and the control sample (RQ = 3.92), the cells treated with apocynin alone (RQ = 0.69), and the HRV-infected cells (RQ = 7.02) ($p < 0.05$; Fig. 1A). However, infected PBMCs displayed significant effect of apocynin only when compared to apocynin alone, without infection (RQ = 2.4 *versus* RQ = 0.57 respectively, $p < 0.05$ Fig. 1B).

The effect of rhinovirus infection on TGF-β protein expression was confirmed in PBMCs and fibroblasts (OD units = 181.27 *vs.* 154.11 and 179.12 *vs.* 129.59, respectively) ($p < 0.05$, Fig. 2A and B). Moreover, protein expression decreased to a lower level in infected cells following apocynin treatment, compared to untreated infected cells and uninfected cells treated with apocynin (OD units = 168.03 *vs.* 179.12 and *vs.* 128.39, respectively) ($p < 0.05$, Fig. 2A and Fig. 7).

2.2. TSLP increases TGF-β1 mRNA expression in fibroblasts PBMC

TSLP caused an increase in TGF-β1 mRNA expression; however, this increase was significant in PBMC but not in NHLF (RQ = 6.11, $p < 0.05$, RQ = 2.47, $p > 0.05$ respectively, Fig. 1). Under conditions of viral infection, TSLP enhanced the effect of HRV in PBMCs (RQ = 6.99 *vs.* 5.86), but surprisingly, in infected fibroblasts, TSLP decreased TGF-β1 expression in comparison to virus alone (RQ = 3.46 *vs.* 7.02, $p < 0.05$, Fig. 1A).

Additionally, in PBMCs, an increased TGF-β protein level was observed in infected cells in the presence of TSLP (OD units = 189.65) compared to controls (OD units = 154.11) ($p < 0.05$, Fig. 2B and Fig. 7).

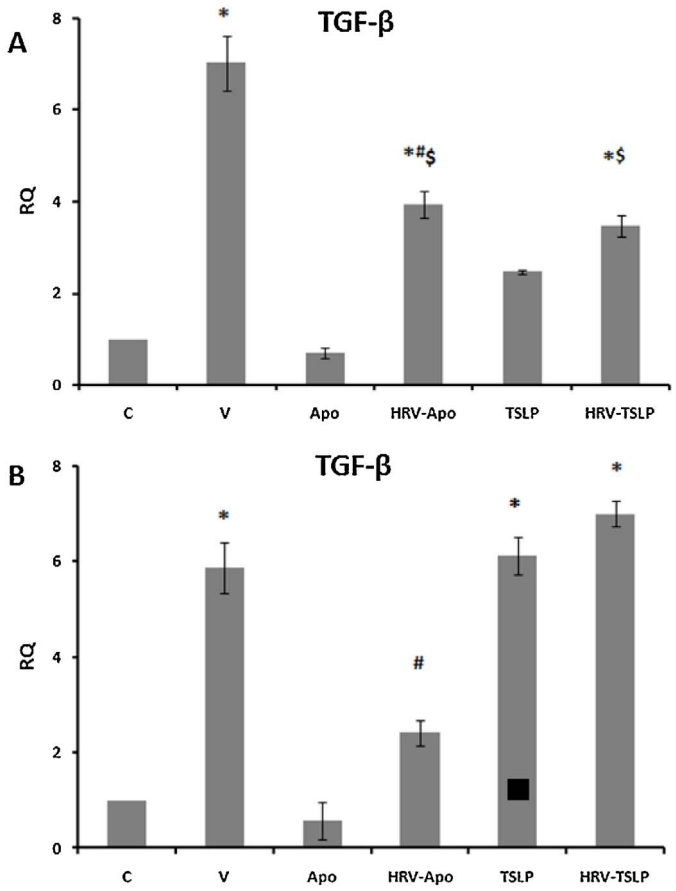

Fig. 1. Effect of apocynin (Apo), TSLP treatment and rhinovirus-16 (HRV) infection on the expression of TGF-β mRNA in NHLF (A) and PBMC (B).
Human fibroblasts and PBMCs were incubated with rhinovirus-16 for 48 h and then either apocynin or TSLP were added for the next 24 h. The results of the qPCR are presented as relative expression in relation to β-actin. Rhinovirus increased TGF-β mRNA expression and the effect was inhibited by apocynin. TSLP increased mRNA expression and enhanced the effect of HRV16. $^{*}p < 0.05$ *vs.* control sample, $^{\#}p < 0.05$ *vs.* apocynin/TSLP, $^{\$}p < 0.05$ *vs.* Rhinovirus 16. Data presented as Relative Quantity ± SD.

2.3. Apocynin enhances TIMP-1 expression on the mRNA level, but not the protein level.

The effect of apocynin on TIMP-1 expression in NHLF cells was statistically significant (RQ = 5.45, $p < 0.05$, Fig. 3A). Moreover, apocynin effect under conditions of viral infection was the same as in infected cells without apocynin (RQ = 5.14 *vs.* 2.23, $p < 0.05$ Fig. 3A). No such effect was observed in PBMCs (Fig. 3B). Surprisingly, the ELISA test did not confirm the above results: no significant changes were observed after apocynin administration in PBMC nor in NHLF (Fig. 4A and B).

2.4. TSLP has no significant effect on TIMP-1 expression in PBMC

TSLP incubation resulted in decreased TIMP-1 mRNA expression in fibroblasts, but this change was found to be insignificant (RQ = 0.38). Nevertheless, TSLP caused an insignificant decrease of TIMP-1 expression in HRV-infected cells (RQ = 0.88) when compared to HRV alone (RQ = 2.2) ($p > 0.05$, Fig. 3A). TSLP was not found to have any effect in PBMC (Fig. 3B) on the protein level (Fig. 4B). Surprisingly, a significant increase was observed in HRV-infected fibroblasts after TSLP incubation (10.12 ng/ml) compared to the control sample (5.94 ng/ml), but also in comparison to TSLP alone (4.69 ng/ml), or HRV (6.36 ng/ml) ($p < 0.05$), Fig. 4A).