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Synergistic inflammatory signaling in airway epithelial cells: control of expression levels of protease-activated receptors and interleukin-8 release

Abstract

Asthma is a chronic inflammatory disease of the airways in which besides migratory cells of the immune system, many resident cells like smooth muscle cells, fibroblasts and epithelial cells play important roles. The airway epithelium acts not only as a physical barrier for inhaled infectious stimuli but also actively participates in acute and chronic inflammatory reactions by releasing pro- and anti-inflammatory mediators. Exposure of epithelial cells to deleterious factors, like allergens, bacteria, pollutants, and to endogenous proinflammatory factors, triggers defence mechanisms by modulation of expression and secretion of different bioactive molecules such as lipid mediators, cytokines, extracellular matrix proteins. It has been shown that the release of those agents is frequently mediated via activation of protease-activated receptors (PARs). PARs belong to the superfamily of G-protein coupled receptors with a 7-trans-membrane domain structure. They are activated by proteolytic cleavage of their N-terminus. For many potential PAR activators in airways elevated activity has been observed during chronic inflammation. Among those proteases are thrombin, tryptase, human airway trypsin-like protease (HAT) as well as proteases from airborne allergens. However, there is still limited information concerning the question which particular factors are responsible for the alteration of PAR expression and susceptibility in lung epithelial cells.

The findings of the present study are as follows:

1. Using RT-PCR, immunocytochemistry and Ca^{2+} mobilization measurements, we demonstrated that the airway epithelial cell line A549 expresses PAR-1, PAR-2, and PAR-3. Short-term stimulation of these cells with thrombin, trypsin, and activating peptides of PAR-1, PAR-2, but not PAR-3 and PAR-4, induced a transient rise of $[Ca^{2+}]_i$.

2. We showed that cationic and anionic trypsin induce Ca^{2+} mobilization in these cells. Mesotrypsin displays no effect on $[Ca^{2+}]_i$ rise. Furthermore, from desensitization study using PAR-2 AP we conclude that PAR-2 is substrate for cationic and anionic trypsin isoforms in human airway epithelial cells.

3. We evaluated the influence of inflammatory mediators LPS, TNF-α, IL-8 and PGE_2 on PAR expression level in A549 cells. We also investigated the influence of continuous PAR activation on PAR expression and release of the proinflammatory chemokine IL-8. We employed three different cells, two airway epithelial cell lines, A549 and HBE cells and primary airway epithelial cells (HSAEC). The bacterial endotoxin LPS after 4 h of stimulation

up-regulated PAR-2 expression 2-fold, but the effect disappeared after 24-h-term stimulation. TNF-α up-regulated PAR-1, -2 and -3 expression after 24 h of incubation. Exposure of the cells to Il-8 slightly decreased PARs mRNA level, mostly PAR-2. Similarly PGE_2 down-regulated PAR expression after 10 h of stimulation, whereas after 16 h PAR-2 mRNA level was up-regulated.

4. Continuous activation of PARs by exposure of epithelial cells to PAR-1 and PAR-2 agonists increased the PAR-1 expression level. The PAR-2 agonist exhibited higher potency than PAR-1 activators. However, in epithelial cells the combined incubation with LPS and PAR agonists abrogated the PAR-1 up-regulation induced by a single stimulus. Stimulation with PAR-1 or PAR-2 agonists also up-regulated the PAR-2 expression level (2.7-fold) that was higher than the effect of PAR agonists on PAR-1 level. In contrast to PAR-1, the PAR-2 level remained elevated under concomitant stimulation with LPS and PAR-2 agonist. PAR-3 mRNA level in A549 cells remained unaffected upon continuous PAR activation, whereas combined stimulation with PAR agonists and LPS resulted in slight down-regulation of PAR-3 level after 24 h.

5. Activation of PAR-2, but not of PAR-1 caused production of IL-8 from the epithelial cell lines. This effect was mediated in A549 cells by c-jun N-terminal kinase (JNK) and extracellular signal regulated kinase-1/2 (ERK1/2). We found synergistic modulation by PAR-2 agonist and LPS of the IL-8 synthesis and release both in the epithelial cell line and in primary epithelial cells.

6. PAR agonists induced also expression of the immunomodulatory cytokine TGF-β1. Simultaneous application of PAR agonists together with LPS attenuated or completely abolished the TGF-β1 up-regulation induced by PAR agonists alone.

7. With the help of HEK-293 cells expressing PAR-3, we demonstrated for the first time, that PAR-3 is involved in thrombin-mediated ERK1/2 activation and release of IL-8. Furthermore, generation of intracellular signals by PAR-3, such as enhancement of cytokine synthesis, does not require co-activation of PAR-1 or PAR-4.