

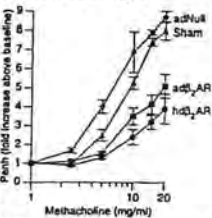
ADENOVIRAL-MEDIATED OVEREXPRESSION OF A β_2 -ADRENERGIC RECEPTOR IN THE BRONCHIAL EPITHELIUM ATTENUATES AIRWAY REACTIVITY IN MICE. P. Factor, Y. Wang, I. Sayeed, L. Shin, J. Burhop, and V. Dumasiu. Pulmonary and Critical Care Medicine, Evanston Northwestern Healthcare, Evanston, IL 60201, Northwestern University Medical School, Chicago, IL.

We have hypothesized that airway epithelial β_2 -adrenergic receptors (β_2 AR) are important modulators of airway smooth muscle tone and that β_2 AR overexpression in the bronchial epithelium can attenuate methacholine-induced airway reactivity. To test our hypothesis we infected male C57Bl6 mice with 1×10^{10} viral particles of 1st generation and fully-deleted adenovectors that express a human β_2 AR cDNA (ad β_2 AR and hd β_2 AR, respectively). These mice were compared to controls given vehicle alone (sham) or a 1st generation adenovirus that contains no cDNA (adNull). Virus was delivered via tracheotomy in 20 μ l of PBS 3 days prior to study. Transgene expression, measured via immunohistochemistry using a human β_2 AR antibody, was confined to the bronchial epithelium and was noted in =80% of airways.

The dose of aerosolized methacholine that increases airway resistance by 200% above baseline (EC_{200}) was measured using a whole body barometric plethysmograph (Buxco). The EC_{200} in ad β_2 AR mice (8.50 ± 0.35 mg/ml, $p=0.01$ vs sham) was up to 250% greater than sham and adNull controls (EC_{200} : 5.75 ± 0.58 and 3.44 ± 0.81 mg/ml, respectively). Attenuation of airway reactivity was most pronounced in mice infected with hd β_2 AR (EC_{200} : 10.01 ± 4.5 mg/ml, $p<0.002$ vs. all other groups). Airway reactivity in adrenalectomized mice infected with β_2 AR was not different from sham infected control.

These results suggest that increasing the number of β_2 ARs in the bronchial epithelium attenuates methacholine-sensitivity via improved responsiveness to endogenous catecholamines. These effects are most pronounced in mice infected with an adenovector that does not express any adenoviral genes (hd β_2 AR). These studies indicate that epithelial β_2 ARs can regulate bronchomotor tone and that genetic "receptor" therapies that increase β_2 AR function in airway epithelial cells may prove useful for the treatment of asthma.

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PROTEINASE-ACTIVATED RECEPTOR-2: EXPRESSION BY PULMONARY EPITHELIAL CELLS AND EFFECT OF SERINE PROTEINASES. S. Dulon, C. Candé, D. Pidard and M. Chignard. Défense innée et Inflammation, Inserm U485, Institut Pasteur, Paris, France.

Protease-activated receptors (PAR) are a novel emerging family of G protein-coupled receptors. They are seven-transmembrane domain receptors which are cleaved and activated by serine proteinases. Trypsin cleaves the aminoterminal extracellular extension of human PAR-2 creating a new amino terminus starting by SLIGKV which binds intramolecularly and activates the receptor. The synthetic peptide corresponding to this tethered ligand domain activates PAR-2 without the need for receptor cleavage. Recent studies have shown that human pulmonary epithelial cells can be activated through PAR-2 to release inflammatory molecules such as MMP-9, GM-CSF, and eotaxin.

In the present study we provide evidence that PAR-2 is expressed by human alveolar (A549) and bronchial (16-HBE) epithelial cell lines in culture, based on the analysis of mRNA by RT-PCR and protein by flow cytometry. We confirm that its activation by trypsin and by the activating peptide, SLIGKV-NH₂, leads to intracellular calcium mobilization. It is well recognized that during inflammation, lungs are burdened by polymorphonuclear neutrophils and that their serine proteinases, elastase and cathepsin G, are released in the airspaces. We here demonstrate that these proteinases are unable to activate PAR-2 but rather disable it. Thus, elastase and cathepsin G prevent any further activation of the cells by trypsin and as such have the potential to suppress the PAR-2-dependent production of inflammatory mediators by pulmonary epithelial cells.

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HUMAN NEUTROPHIL ELASTASE (HNE) INDUCES MUC5AC PRODUCTION VIA ACTIVATION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR): ROLE OF TRANSFORMING GROWTH FACTOR-ALPHA (TGF- α)

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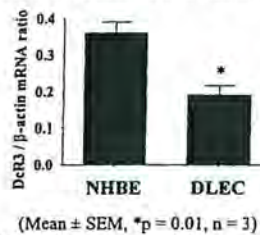
[Introduction] HNE is implicated in hypersecretory diseases of airways. To clarify mechanisms linking proteolytic effects of HNE and mucin production, we studied the effect of HNE on mucin MUC5AC production and the role of the EGFR ligand, TGF- α , in cultured airway epithelial cells. [Methods] NCI-H292 cells grown in RPMI 1640 medium were serum-starved and stimulated with HNE. MUC5AC, TGF- α , and EGFR protein production were examined by ELISA or immunocytochemistry. EGFR activation was analyzed by Western blotting. [Results] Incubation of NCI-H292 cells with HNE (10^{-7} M) for 30 min resulted in increased MUC5AC production 24 h later. This induction was preceded by EGFR phosphorylation and inhibited by EGFR tyrosine kinase inhibitors (AG1478, 10^{-6} M; BIBX1522, 10^{-6} M) or by a mitogen-activated protein kinase kinase inhibitor (PD98059, 10^{-6} M), implicating an EGFR signaling cascade. Elastase inhibitors (ICI 200,355, 10^{-6} M; SLPI, 10^{-6} M) prevented HNE-induced MUC5AC production, implicating a proteolytic action of the serine active site of HNE. HNE-induced MUC5AC production was inhibited by a neutralizing EGFR antibody (4 μ g/ml) and by a TGF- α antibody (4 μ g/ml), implicating EGFR-ligand binding in the response. HNE released TGF- α from NCI-H292 cells, an effect that was inhibited by ICI 200,355. Furthermore, HNE induced TGF- α and EGFR protein production. [Conclusion] HNE stimulates mucin MUC5AC production via an EGFR signaling cascade involving protease-induced TGF- α release and production, EGFR up-regulation, and EGFR activation in airway epithelial cells.

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DIFFERENTIAL EXPRESSION OF DECOY RECEPTOR 3 ALONG THE LUNG EPITHELIAL SURFACE. M. Nakamura, G. Matute-Bello, C.W. Frevert, W.C. Liles, O. Kajikawa, T.R. Martin. Pulmonary and Critical Care Medicine, Seattle VA Medical Center and University of Washington, Seattle, WA.

[Introduction: We previously reported that human distal lung epithelial cells (DLEC) were more sensitive to sFasL than proximal lung epithelial cells (normal human bronchial cells, NHBE) even though membrane surface Fas expression was similar in proximal and distal lung epithelial cells. The decoy receptor 3 (DcR3) is a member of the TNF receptor family that competes with Fas for FasL binding and inhibits FasL induced apoptosis. Various intracellular proteins (Bcl-2, Bcl-xL/S, Bax, BID, IAP-1, IAP-2, XIAP, and FLIP) are known to regulate the Fas/FasL pathway. Goal: In the present study, we investigated the differential expression of mRNA for DcR3 and the intracellular proteins, Bcl-2, Bcl-xL/S, Bax, BID, IAP-1, IAP-2, XIAP, and FLIP in NHBE and DLEC.

[Methods: The mRNA expression of these proteins in NHBE and DLEC was analyzed using semi-quantitative RT-PCR. The expression was corrected by β -actin. Results: Only DcR3 mRNA expression was significantly increased in NHBE in comparison with SAEC at 30 cycles of amplification (Figure). Conclusions: This result suggests that increased expression of DcR3 in proximal lung epithelial cells may protect cells from Fas-induced cell death and promote cell survival in NHBE when compared to DLEC.



(Mean \pm SEM, * $p = 0.01$, $n = 3$)
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DEXAMETHASONE INDUCE β -CATENIN SIGNALING PATHWAY IN HUMAN BRONCHIAL EPITHELIAL CELLS. N. Carayol, I. Yachier, A.M. Campbell, P. Godard, J. Bousquet, P. Chané. INSERM U454, CHU Montpellier, France.

Cellular cohesion is hallmark of bronchial epithelium. We found that TNF- α and dexamethasone modulate E-cadherin and β -catenin (β -cat) expression in HBEC. Dexamethasone inhibits the cadherin and catenin decrease induced by TNF- α and induces β -cat nuclear translocation in human bronchial epithelial cell (Carayol et al.). The fate of HBEC including cohesion and apoptosis might be related to these processes.

Aim: β -cat has been shown to form a complex with the LEF/TCF transcription factors family. In the present study, we assessed the relationship between the nuclear translocation of Tcf-4, the β -cat/Tcf-4 complex formation and the turnover of HBEC by evaluating proliferation and apoptosis which have been shown to be mediated by the β -cat/Tcf complex.

Methods: SAEC cells were stimulated with dexamethasone \pm TNF- α or with dexamethasone alone, for 3, 6, 12 and 24h. We investigated the Tcf-4 nuclear translocation by immunofluorescence, the induction of β -cat/Tcf-4 complex by immunoprecipitation, the inhibition of apoptosis by measuring caspase-3 activation with a colorimetric assay system and the induction of proliferation by 3 H thymidine incorporation.

Results: We found that dexamethasone induced the Tcf-4 nuclear translocation and the formation of β -cat/Tcf-4 complex. Dex induced 50% inhibition ($p<0,05$) of caspase-3 activity at 3, 12 and 24h, but failed to inhibit apoptosis at 6h: this may be related to the inhibition of NF- κ B pathway. Dex also induce a 3 fold ($p<0,05$) induction of proliferation on 24h, with a maximum of 5 fold at 12h ($p<0,05$) even though proliferation was less marked at 24h.

Conclusion: We found that dexamethasone effectively induced β -cat signaling pathway. This signaling pathway may explain the bronchial epithelial cohesion induced by dexamethasone, inducing proliferation and inhibiting apoptosis.

EPITHELIUM-DEPENDENT RELAXATION RESPONSES OF GUINEA-PIG ISOLATED, PERFUSED TRACHEA TO HYPERTONIC SOLUTION INVOLVES CARBON MONOXIDE (CO). *†J.S. Fedan, *J. Dowdy, †M.J. Reasor, †M.R. Van Scott, and †R.A. Johnston. *PPRB, HELD, NIOSH, Morgantown, WV 26505, †West Virginia University, Morgantown, WV 26506; ‡East Carolina University, Greenville, NC 27858; and §Harvard University, Boston, MA 02115.

Application of hypertonic solution to the mucosal surface of the guinea-pig isolated, perfused trachea (IPT) elicits epithelium-dependent relaxation of airway smooth muscle via epithelium-derived relaxing factor (EpDRF), the identity of which is unknown. The purpose of this study was to examine whether CO mediates smooth muscle relaxation. After contracting the smooth muscle with serosally-applied methacholine (3×10^{-7} M), the PT was challenged with mucosally-administered D-mannitol (D-M; 120 - 160 mosM), a non-permeant osmolyte. The effects of inhibitors on the response to D-M were examined. Neither cyclooxygenase inhibition with indomethacin (3×10^{-6} M; 30 min) nor inhibition of nitric oxide (NO) synthase with N ω -nitro-L-arginine methyl ester (L-NAME; 10^{-4} M; 30 min) affected the response to D-M, indicating that neither prostanooids nor NO are involved. Upon addition of the CO scavenger, hemoglobin (Hb; 10^{-6} M; 30 min), the IPT developed small contractile responses. In the presence of Hb the relaxation to D-M was inhibited significantly. Likewise, after incubation with the heme oxygenase inhibitor, zinc (II) protoporphyrin IX (10^{-4} M; ZnPP; 1 h), the relaxation response to D-M was inhibited significantly. Neither Hb nor ZnPP abolished completely the relaxation response to D-M. These results suggest that epithelium-derived CO participates in the hypertonicity-induced relaxation of the trachea.

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