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Protease-activated receptor-2 (PAR-2) is a weak enhancer of mucin secretion by human bronchial epithelial cells in vitro

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Abstract

PAR-2, a member of a family of G-protein-coupled receptors, can be activated by serine proteases via proteolytic cleavage. PAR-2 expression is known to be upregulated in respiratory epithelium subsequent to inflammation in asthma and chronic obstructive pulmonary disease (COPD). Since these diseases also are characterized by excessive mucus production and secretion, we investigated whether PAR-2 could be linked to mucin hypersecretion by airway epithelium. Normal human bronchial epithelial (NHBE) cells in primary culture or the human bronchial epithelial cell lines, NCI-H292 and HBE-1, were used. NHBE, NCI-H292, and HBE-1 cells expressed prominent levels of PAR-2 protein. Short-term (30 min) exposure of cells to the synthetic PAR-2 agonist peptide (SLIGKV-NH_2) elicited a small but statistically significant increase in mucin secretion at high concentrations (100 μM and 1000 μM), compared to a control peptide with reversed amino acid sequence (VKGILS-NH_2). Neither human lung tryptase nor bovine pancreatic trypsin, both PAR-2 agonists, affected NHBE cell mucin secretion when added over a range of concentrations. Knockdown of PAR-2 expression by siRNA blocked the stimulatory effect of the AP. The results suggest that, since PAR-2 activation only weakly increases mucin secretion by human airway epithelial cells in vitro, PAR-2 probably is not a significant contributor to mucin hypersecretion in inflamed airways.

Keywords: PAR-2; Mucin; Airway; Secretion

1. Introduction

Several proteases, including mast cell tryptase (MCT), matrix metalloproteinases (MMP), bacterial protease, and neutrophil elastase have been implicated in the pathogenesis of inflammatory airway diseases (Cairns & Walls, 1996; Kon et al., 1999; Nakamura, Yoshimura, McElvaney, & Crystal, 1992; Ohno et al., 1997). Many of these diseases, such as asthma, chronic bronchitis, chronic obstructive pulmonary disease (COPD) and cystic fibrosis, are characterized by enhanced production and hypersecretion of mucin which contributes to airway obstruction and disease complications. Interestingly, many of the above proteases, whose levels may increase in these diseases, also can provoke production and secretion of mucin by airway epithelium. Mechanism(s) by which these proteases stimulate mucin secretion are not known.

Protease-activated receptors (PARs) are a family of G-protein-coupled receptors found in several cell types (Dery, Corvera, Steinhoff, & Bunnett, 1998). Currently, four PAR family members have been identified, cloned and designated as PAR-1 to -4. PAR-2 is expressed in the respiratory and gastrointestinal tracts (Cocks & Moffatt, 2001; D'Andrea et al., 1998; Kawabata et al., 2001; Knight et al., 2001). It can be activated via proteolytic cleavage by serine proteases, especially trypsin

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and tryptase. Activation of PAR-2 has been implicated in pro-inflammatory processes in various tissues and cells (Miike, McWilliam, & Kita, 2001; Nystedt, Ramakrishnan, & Sundelin, 1996; Steinhoff et al., 2000), however, its potential role(s) in the pathogenesis of inflammatory airway diseases has not been elucidated. It is known that overexpression of PAR-2 may be induced under pathogenic circumstances, as enhanced PAR-2 expression is observed in respiratory epithelium of asthmatics and COPD patients (Cocks & Moffatt, 2001; Knight et al., 2001; Miotto et al., 2002). Although there are no reports linking PAR-2 activation to the actual secretory process, it has been shown that PAR-2 is involved in secretion of mucin by epithelium from the stomach and salivary glands in rodents (Kawabata et al., 2000; Kawabata, Nishikawa, Kuroda, Kawai, & Hollenberg, 2000; Kawabata et al., 2001).

In this study, we hypothesized that proteases associated with airway inflammation, such as human lung tryptase and human neutrophil elastase (HNE), may provoke mucin secretion by human airway epithelium via activation of PAR-2 receptors on the cell surface. To look at this question, we examined whether or not direct activation of PAR-2 on human airway epithelial cells in vitro could stimulate mucin secretion. The results indicate that activation of PAR-2 receptors on airway epithelial cells enhances secretion of mucin, but only weakly and only in response to relatively high concentrations of PAR-2 agonists. Thus, it would seem that PAR-2 activation alone is not an important factor in enhanced mucin secretion in inflamed airways.

2. Materials and methods

2.1. Materials

Normal human bronchial epithelial (NHBE) cells were purchased from Lonza (Walkersville, MD), as was bronchial epithelial cell growth medium (BEGM) and supplemental factors for NHBE cells. NCI-H292 cells were purchased from American Type Culture Collection (ATCC, CRL-1848, Manassas, VA). The immortalized normal bronchial epithelial cell line HBE1 was a gift from Dr. Reen Wu (University of California, Davis, CA). Transwell culture inserts were from Corning Inc. (Corning, NY), and rat tail collagen type I was from BD Biosciences (San Jose, CA). Dulbecco's modified Eagle's medium (DMEM) and RPMI 1640 medium were from Mediatech, Inc. (Herndon, VA). Bovine serum albumin (BSA) was purchased from Millipore (Billerica, MA), nystatin from Amresco (Solon, OH), all-*trans* retinoic acid from Sigma (St. Louis, MO), and cholera toxin from List Biological Labs (Campbell, CA). Bovine pituitary extract (BPE) was prepared by a modification of the method of Bertolero, Kaighn, Gonda, and Saffiotti (1984) using pituitaries purchased from Pel-Freez (Rogers, AR). Trans-Blot transfer membranes were from Bio-Rad (Hercules, CA), goat polyclonal anti-PAR-2 antibody, mouse monoclonal anti-PAR2 (SAM11) antibody, HRP-conjugated donkey anti-goat IgG, and horseradish peroxidase (HRP) -conjugated goat anti-mouse IgG were from Santa Cruz (Santa Cruz, CA). An enhanced chemiluminescence detection system was purchased from Amersham Biosciences Inc. (Pittsburgh, PA). ChromPure goat IgG was from Jackson Immunoresearch (West Grove, PA). DAB Chromogen was from Dako (Carpinteria, CA), Permount from Fisher (Pittsburgh, PA), Versene from Invitrogen (Carlsbad, CA), modified Harris Hematoxylin from VWR (West Chester, PA), and Cytospin2 from Shandon (Pittsburgh, PA). The PAR-2 activating peptide AP (SLIGKV-NH_2) and the reverse peptide control RP (VKGILS-NH_2) were purchased from Genemed Synthesis Inc. (South San Francisco, CA), and human lung tryptase was purhcased from U.S. Biologicals (Swampscott, MA). Endotoxin-free HNE purified from human sputum and the HNE inhibitor, chloromethyl ketone-modified tetrapeptide (CMK) were from Elastin Products Company (Owensville, MO). 17Q2 antibody was from Covance corporation (Berkeley, CA). Gastric MUC5AC mucin antibody (45M1) was from Neo-Markers (Fremont, CA). CytoTox 96 nonradioactive cytotoxicity assay kits were from Promega (Madison, WI). All other chemicals and reagents were from Sigma (St. Louis, MO).

2.2. Cell culture

2.2.1. NHBE cells

Expansion, cryopreservation, and culture of NHBE cells in air/liquid interface were performed as described previously (Krunkosky et al., 2000). Briefly, air/liquid interface culture was initiated by seeding passage-2 NHBE cells in Transwell® inserts coated with rat tail collagen type I at a density of 2×10^4 cells/cm^2. The culture medium was a 1:1 mixture of BEGM and DMEM containing 130 μg/ml BPE, 5×10^{-8} M all-*trans* retinoic acid, 1.5 μg/ml BSA, 20 U/ml nystatin, 0.5 ng/ml human recombinant epidermal growth factor (hEGF), 0.5 μg/ml hydrocortisone, 5 μg/ml insulin, 10 μg/ml transferrin, 0.5 μg/ml epinephrine, 6.5 ng/ml triiodothyronine, 50 μg/ml gentamicin, and 50 μg/ml amphotericin-B. Cells were cultured submerged in medium in a humidified 95% air, 5% CO_2 environment