Enhanced Expression of Myristoylated Alanine – Rich C Kinase Substrate (MARCKS) in Airway Epithelium of an Animal Model of Allergic Airway Inflammation and Human Patients with COPD

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ABSTRACT

The ovalbumin (OVA)– sensitized mouse is a well-characterized model of allergic airway inflammation resembling human COPD. We and others have shown previously that these mice develop goblet cell hyperplasia and metaplasia, that they hypersecrete mucin in response to stimulation, and that mucin hypersecretion is attenuated by pretreatment with aerosolized or intratracheally administered peptides of MARCKS protein, namely the MANS peptide or its truncated form, Bet30-40AM. We have also shown that ovalbumin sensitized mice were immunized subcutaneously with OVA and later challenged with OVA in the lungs of sensitized animals. Those animals challenged with OVA showed a marked increase in mucin secretion compared to mice injected with saline. We have also shown that aerosolized ovalbumin (OVA) was instilled into each nostril alternately over 2 days. To test whether this was more effective than a single dose, we administered aerosolized methylcholanthrene (MCH) for 72 hrs. After OVA challenge, we injected saline or MANS into the nasal passages of mice instilled with OVA or saline. The right lung was inflated with a 50% vol/vol mixture of Tissue-Tek OCT and was frozen on dry ice in a plastic mold filled with Tissue-Tek OCT. The left lung was subsequently sectioned at 8 um and stained for histological analysis. To perform real time PCR, the lungs of sensitized and control animals were separated (arrows) from the trachea using Laser Capture Micro-dissection, we identified and, applying Laser Capture Microdissection and real time PCR, MARCKS and MUC5AC gene expression between normal and treated mice (P=0.018/MARCKS and 0.023/MUC5AC). Using LCM and real time PCR, detection and comparison of MARCKS expression in normal and inflammatory lungs as seen shows increases in expression of MARCKS and MUC5AC in airway epithelial cells of OVA mice.

OBJECTIVES

- To detect MARCKS and mucin gene and protein expression in an established asthma model.
- To investigate the relative gene expression at different anatomic sites in vivo by applying Laser Capture Microdissection and real time PCR.
- To determine the applicability in vivo of peptide application to inhibit mucin hypersecretion during a pathological process.

METHODS

Animal model
Six to eight-week-old BALB/c mice between 16 and 20 g were housed in accordance with the institutional guidelines. To induce mucin hypersecretion, mice were sensitized to ovalbumin (10 μg ovalbumin, grade V, 2:2:12 mg alum in saline, pH 7.4) in Sigma, St. Louis, MO) according to weekly intraperitoneal injections for 4 weeks. Sensitized mice were then challenged with an aerosol of 2.5% (wt/vol) ovalbumin in 0.9% saline for 3 days. At the end of the culture period, the bronchoalveolar lavage (BAL) fluid from the trachea was used as a bronchoalveolar lavage (BAL) fluid from the trachea was used as a source of airway goblet cells as seen with MANS pretreatment.

RESULTS

Mucin secreted in mouse BAL

Processing of Lung Tissue

Mice were euthanized with an intraperitoneal injection of ketamine and xylazine, and BALF was obtained by cannulating the trachea and instilling the lungs with two 0.75 ml aliquots of ice-cold Dulbecco’s PBS (Sigma, St. Louis, MO). Then lungs were perfused with saline and the right lung was inflated with a 50% vol/vol mixture of Tissue-Tek OCT and was frozen on dry ice in a plastic mold filled with Tissue-Tek OCT. For histologic analysis, the left lung was subsequently sectioned at 8 um and stained with hematoxylin and eosin. The right lung was fixed at 4% formalin for histology.

Laser Capture Microdissection and Real-time PCR

Specific anatomic regions of the lungs were collected via a laser micromanipulator (LCM) and real time PCR. Both MARCKS and MUC5AC gene expression were increased by 3.5 fold in BALF cells, similarly in ovalbumin (OVA)-sensitized mice. However, only an increase in bronchial MARCKS mRNA was observed, but the peptide did not affect the increase in MUC5AC expression. In contrast to airways, LCM of parenchymal regions of the lung showed no increase in mRNA levels of MARCKS in OVA-treated animals. Interestingly, epithelial cells isolated from mice with COPD also showed significantly increased levels of MARCKS in vivo, identifying MUC5AC as a potential biomarker of airway inflammation. The results suggest that message and protein levels of MARCKS are increased in epithelial cells in both an animal model of allergic airway inflammation and human patients with COPD, perhaps related to mucin hypersecretion and hypersecretion characterizing this disease, since MARCKS is an intracellular regulator of mucin secretion. The mechanism by which the MANS peptide attenuates mRNA levels of MARCKS in airway epithelium requires further investigation.

CONCLUSIONS

1. Using Laser Capture Microdissection and real time PCR, it is possible to develop a quantitative analysis of expression of MARCKS and mucin related genes in different anatomic sites of the lung in vivo.
2. The MANS peptide appears to attenuate MARCKS mRNA expression in vivo, but does not seem to affect MUC5AC mRNA expression.
3. The results suggest that message and protein levels of MARCKS are increased in epithelial cells in both an animal model of allergic airway inflammation and human patients with COPD. This is perhaps related to mucin cell hyperplasia and hypersecretion, since MARCKS is an intracellular regulator of airway mucin secretion.

ACKNOWLEDGEMENTS: This work was supported by NHLBI grant R37 HL36982.