MARCKS-related peptide modulates in vivo the secretion of airway Muc5ac

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Foster WM, Adler KB, Crews AL, Potts EN, Fischer BM, Voynow JA. MARCKS-related peptide modulates in vivo the secretion of airway Muc5ac. Am J Physiol Lung Cell Mol Physiol 299: L345–L352, 2010. First published June 11, 2010; doi:10.1152/ajplung.00067.2010.—In a mouse model of neutrophil elastase-induced bronchitis that exhibits goblet cell metaplasia and inflammation, we investigated the effects of intratracheal instillation of the MANS peptide, a peptide identical to the NH2 terminus of the myristoylated alanine-rich C kinase substrate (MARCKS) on mucin protein airway secretion, inflammation, and airway reactivity. To induce mucus metaplasia in the airways, male BALB/c mice were treated repetitively with the serine protease, neutrophil elastase, on days 1, 4, and 7. On day 11, when goblet cell metaplasia was fully developed and profiles of proinflammatory cytokines were maximal, the animals were exposed to aerosolized methacholine after intratracheal instillation of MANS or a nonsense control peptide (RNS). MANS, but not RNS, attenuated the methacholine-stimulated secretion of Muc5ac (50% reduction). Concurrently, elastase-induced proinflammatory cytokines typically recovered in bronchoalveolar lavage (BAL) fluid, including KC, IL-1, IL-6, MCP-1, and TNFα, were reduced by the MANS peptide (mean levels decreased 50–60%). Secondary to the effects of MANS on mucin secretion and inflammation, mechanical lung function by forced oscillation technique was characterized with respect to airway reactivity in response to cumulative aerosol stimulation with serotonin. The MANS peptide was also found to effectively attenuate airway hyperresponsiveness to serotonin in the airway hypersecretory model. Collectively, these findings support the concept that even in airway epithelia remodeled with goblet cell metaplasia and in a state of mucin hypersecretion, exogenous attenuation of function of MARCKS protein via the MANS peptide decreases airway mucin secretion, inflammation, and hyperreactivity.

Muc5ac, myristoylated alanine-rich C kinase substrate; airway hyperresponsiveness, elastase; serotonin

A MUCUS FLUID LAYER COVERS the airway surface and is essential to defense and protection of the respiratory epithelium from inhaled xenobiotic materials. Airway mucus is composed of serous secretions and glycoproteins known as mucins. Intracellular mechanisms controlling the process of mucin secretion by airway epithelial cells are only now beginning elucidated. Current understanding is that within respiratory goblet cells, mucins are synthesized and stored in cytoplasmic membrane-bound granules. On appropriate stimulation, the granules translocate to the apical borders of the cell, and, following fusion of granule membranes with the cellular plasma membrane, mucins are released onto airway epithelial surfaces via an exocytotic process (4, 13). Nine mucins are currently recognized to be expressed in normal respiratory tract tissues (MUC1, 2, 4, 5AC, 5B, 7, 8, 13, and 19; Ref. 16). Inflammatory and obstructive airway diseases, including bronchitis, asthma, bronchiectasis, and cystic fibrosis, are characterized by excessive mucus production and mucin secretion that contribute to narrowing of airway lumens, exacerbations, and related complications (6, 16). MUC5AC and 5B, the most abundant airway mucins, as well as MUC2 have been shown to be modulated in inflammatory airway diseases (2, 16, 20). Excess mucin mucin secretion may increase susceptibility to infection, disrupt innate defenses that may depend on mucociliary function, and interfere with airflow conduction and laminar airflow within central lung airways, further enhancing airway obstruction.

Standard techniques for morphological examination of the airway epithelium (biopsy samples) demonstrate a correlation between pathological findings and in vivo tests of airway function (14). However, in vivo studies of innate epithelial defenses (such as airway mucociliary clearance) have not been found to tightly correlate with indices of mechanical lung function. In the presence of an abnormally remodeled secretory airway epithelium, capable of excessive synthesis and secretion of mucins, as occurs in obstructive airway diseases, the integration between the physiological events of mucin secretion and airway function have not been apparent. However, in a mouse model of allergen-induced airway epithelial cell mucus metaplasia, we (1) recently reported that inhibition of airway mucin secretion led to improving airflow and reducing obstruction. Blockade of secretion was achieved by inhibiting myristoylated alanine-rich C kinase substrate (MARCKS) protein, which had been demonstrated to be a key molecule regulating mucin secretion by airway epithelium in vitro (10) and in vivo (17). The results (1) support the hypothesis that epithelial mucin hypersecretion may make a substantial contribution to airflow obstruction, and thus selective inhibition of mucin hypersecretion potentially represents an alternative strategy to airflow smooth muscle relaxation to improve airflow.

Previously, we (21) developed a bronchitic mouse model of mucus cell metaplasia that predisposes to larger diameter airways in which the epithelium rapidly remodels into a mucus hypersecretory state. This model permits histological validation of mucus cell metaplasia and is supported by biological and molecular evidence of Muc5ac mRNA and mucin protein expression in lung and airway tissues that occur simultaneously with an increase of proinflammatory cytokines in bronchoalveolar lavage (BAL) fluid. The present report extends our prior molecular, biological, and histological characterization of this model to now include results with respect to pulmonary function abnormalities and utilizes selective inhibition of
MATERIALS AND METHODS

Animals. Male six- to eight-week-old BALB/c mice were purchased from The Jackson Laboratories (Bar Harbor, ME). The mice were housed in plastic shoebox-type cages and were maintained on a 12-h diurnal cycle with food and water provided ad libitum. Experimental protocols were approved by the Institutional Animal Care and Use Committee at Duke University Medical Center and were carried out in accordance with the standards for experimental use of animal species established by the U.S. Animal Welfare Act.

Development of mucus cell metaplasia model and stimulation of mucus secretion. Methods for developing the mucus cell metaplasia model are as previously fully described (21). Briefly, anesthetized mice receive an exogenous dose (50 µg) of human neutrophil elastase (NE; Elastin Products, Owensville, MO) or vehicle [1:1 sodium acetate (NaOAc)-to-glycerol] by oropharyngeal aspiration on days 1, 4, and 7. By day 11 in this model, mucus cell metaplasia and tissue Muc5ac mRNA and protein expression are maximally and significantly increased (21). Therefore, by experimental design, mice on day 11 were lightly anesthetized by inhalation with isoflurane and given by intranasal aspiration 50 µl of 100 µM myristoylated alanine-glycine-terminal sequence peptide (MANS; Genemed Synthesis, San Francisco, CA), scrambled missense control peptide (RNS), or 0.9% NaCl. After 15 min, to induce airway mucus secretion, mice were challenged with methacholine aerosol (60 mM; Sigma-Aldrich, St. Louis, MO) for 3 min in a nose-only aerosol exposure chamber. This concentration of methacholine had been demonstrated previously to induce secretion of airway mucins in mice (1, 17). At this postmethacholine challenge, mice were either further tested for in vitro sensitivity of airflow resistance to cumulative doses of serotonin (Sigma-Aldrich) by aerosol challenge (see In vivo measurement of airflow resistance) or euthanized by CO2 inhalation and immediately underwent collection of BAL fluid. For BAL collection, sterile saline (3 ml) was instilled at 1 ml at a time over a period of 25 cm and retrieved. Return volume was recorded, and was consistently >75% of the instilled volume. BAL fluids were immediately placed onto an ice bed for subsequent separation of cellular and fluid constituents. BAL cells were isolated by centrifugation (500 g × 10 min), and the supernatant was stored at −80°C for assessment of cytokine levels. Cell differential was determined by a cytocentrifuge (Cytospin 3; Shandon, Pittsburgh, PA) and Wright-Giemsa stain (Diff-QuiK Stain Set; Harleco, Gibbstown, NJ). Cell differentials were determined for 200 total cells per slide and expressed as a percentage of total leukocytes. Total and differential cell counts were expressed as means ± SE for each group of animals.

In vivo measurement of airflow resistance. The procedure for in vivo measurement of airflow resistance by flexiVent methodology and forced oscillation are as previously fully described (22). Briefly, mice were anesthetized (60 mg/kg pentobarbitral) intraperitoneally prepared with a tracheal cannula, and then placed on a computer-controlled ventilator (flexiVent; SCIREQ, Montréal, Québec, Canada) at a constant tidal volume of 6–8 ml/kg and a positive end-expiratory pressure of 3 cmH2O. The animals received neuromuscular blockade (0.8 mg/kg pancuronium bromide; Sigma-Aldrich) and were permitted 5 min to adjust to the ventilator. Airway pressure was monitored at a side port of the tracheal cannula, and airway resistance was calculated using pressure and volume data that were generated by applying a 2-s sine-wave volume perturbation to the airway. Changes in airway resistance from baseline were induced by serotonin challenge. Serotonin was dissolved in 0.9% NaCl at increasing concentrations of 10, 25, 50, and 100 mg/ml and administered by aerosol using an ultrasonic nebulizer (UltraNeb 2000; DeVilbiss, Somerset, PA) that was placed inline with the ventilator and set to deliver aerosol for 30 s at a rate of 120 breaths/min. Airway resistance was assessed at baseline and following each serotonin dose every 20 s for 4 min ensuring that the parameters calculated had peaked. The resistance measurements were then averaged at each dose and graphed linearly [average total pulmonary resistance (Rt) cmH2O·ml−1·s−1] along with the initial baseline value. Assessment of airway resistance required ~15 min per mouse from the point at which the mouse received anesthesia and was prepared for surgery until the conclusion of the serotonin challenges, followed by animal euthanasia. Since we had employed methacholine (described above) as the agonist to induce mucus secretion, the potential existed for airway tissues to demonstrate tachyphylaxis to this agonist (18), which would complicate interpretation of whether airway hyperresponsiveness was indeed present in the model. Therefore, by experimental design, we selected serotonin as the provocative agonist to assess airway responsiveness, rather than methacholine, the prototypal agonist usually preferred in mouse studies.

Mucin production and cytokine analyses. BAL samples were analyzed by mouse ELISA using the 45M1 monoclonal antibody (Zymed, Fremont, CA) that specifically reacts with a core peptide epitope on Muc5ac (21), the predominant airway mucin in mice, in a direct capture protocol (15, 16). Samples with unknown mucin concentration were serially diluted and titered against several antibody concentrations from this titer, sample dilutions of 1:100 and antibody dilutions of 1:500 were chosen for all BAL samples analyzed. Briefly, the plates were loaded with 180 µl/well of samples in coating buffer (200 mM carbonate buffer, pH 9.5) and incubated at room temperature for 1 h. The plates were then washed three times with wash buffer (0.05% Tween 20 in PBS) followed by the addition of 200-µl blocking buffer (5% nonfat dry milk in PBS) per well and incubated for an additional 2 h at room temperature. Next, the plates were washed, and 50 µl of 45M1 monoclonal primary antibody, diluted 1:500 in 0.5% blocking buffer, were added to each well for 1 h. Washes were repeated, followed by the addition of 50 µl of goat anti-mouse IgG-HORSE-radish peroxidase (HRP)-conjugated secondary antibody (Zymed) and incubated at room temperature for approximately 15–25 min until the highest standard appeared pale blue. The reaction in the wells was stopped by the addition of 50 µl of 1 M sulfuric acid, which turned the wells yellow. Finally, the optical density of the wells was determined at an excitation wavelength of 450 nm and a reference wavelength of 540 nm on a VersaMax (Molecular Devices) microplate reader. Since there is no MUCSAC mucin standard commercially available at this time, a crude preparation of porcine stomach mucin (Sigma-Aldrich) was used (15), allowing calculation of the concentration of mucin by directly comparing to the known concentration of the porcine stomach mucin standard.

Concentrations of 5 proinflammatory cytokines in BAL fluids were determined by using a commercial multiplex fluorescent bead-based immunoassay (minimum sensitivity 1.0–5.3 pg/ml depending on the cytokine; Millipore). Cytokines analyzed included mouse KC, IL-1β, IL-6, MCP-1, and TNFα. Briefly, duplicate BAL samples (50 µl) and standard concentrations of the respective cytokines were placed in wells in a 96-well microtiter plate. Samples were incubated with 25 µl of anti-mouse multicytokine antibody-immobilized beads, specific for mouse cytokines, and mixed on a plate shaker for 18 h at 4°C. Biotin anti-mouse multicytokine detection antibodies (25 µl) were added to each well as a secondary/detection antibody and incubated at 37°C for 1 h while shaking. Streptavidin-phycocerythrin (25 µl) was added to the wells and incubated with shaking for 30 min at 37°C in the dark. Finally, the beads were washed, resuspended in sheath fluid, and read using a Luminex instrument (Bio-Plex Workstation; Bio-Rad, Hercules, CA) in which a minimum of 50 beads per cytokine for each sample were analyzed.
Lung tissue histochemistry. A histological mucus index, developed previously for the mouse airway (3, 21), was performed on Alcian blue/periodic acid-Schiff (AB/PAS)-stained sections that included both central and peripheral airways. The slides were examined under a ×20 objective, and images were captured with a digital camera. A minimum of 3 representative cross-sectioned airways were imaged per animal. Only airways where the complete circumference of the airway could be visualized and included in the image were analyzed. Airways that opened directly into an alveolar space were not included. The extent of AB/PAS-positive staining in each airway imaged was semiquantitatively determined simultaneously by 2 examiners (B. M. Fischer and J. A. Voynow) who did not have knowledge of treatment conditions, using the following 4-tier grading system: grade 0, no AB/PAS staining; grade 1, ≥25% of the airway epithelium had AB/PAS staining; grade 2, 26–50% of the airway epithelium had AB/PAS staining; grade 3, 51–75% of the airway epithelium had AB/PAS staining; and grade 4, >75% of airway epithelium had AB/PAS staining. This grading system was used to calculate a mucus index score for each mouse group, and results were expressed as means ± SE.

Statistical analysis. Airway physiology measurements were analyzed by ANOVA for multiple comparisons between groups and verified by t-test with Prism (GraphPad Software). Means ± SE were calculated for all indices, and t-tests were obtained from analyses of data sets using Prism.

RESULTS

MANS peptide inhibits NE-induced BAL inflammation. As previously characterized in the NE-induced bronchitis model (21), at day 11 postelastase treatment, the degree of cellular inflammation within lung tissues and collected in BAL fluids parallel each other and are significantly increased. In the present study, mice were challenged, additionally on day 11 with a methacholine aerosol for 3 min and approximately 30–32 min later were killed for collection of BAL. Similar to the earlier report, BAL total cells and cell differentials demonstrated an infiltration of leukocytes in the mice receiving NE treatment compared with vehicle-treated control mice. This characteristic inflammatory phenotype is illustrated in Fig. 1A, and, as shown in treatment with elastase increased total cells in the BAL fluid by 11-fold above the level found for vehicle-treated mice. For elastase-treated mice that were subsequently administered on day 11 the MANS peptide, followed 15 min later by a 3-min aerosol challenge with methacholine, and then killed approximately 30–32 min later, BAL cells were found to be reduced. Thus, in mice given the MANS peptide, the mean level of total BAL cells decreased by 32% compared with mice given RNS or saline. In further analyses of cell differentials, macrophage BAL levels were elevated postelastase treatment, and the MANS-induced reduction in total cells resulted from significant decreases in leukocyte cellularity. For example, in the mice given the MANS peptide, NE-induced increases in polymorphonuclear neutrophil, lymphocytes, and eosinophils fell by 38, 69, and 71%, respectively, compared with mice treated with RNS or saline (Fig. 1B). Additionally, levels of proinflammatory cytokines measured in the BAL fluid were consistently reduced in animals treated with the MANS peptide; these include KC, IL-1β, IL-6, MCP-1, and TNFα. On a percentage basis compared with the RNS scrambled peptide (which had no demonstrable effect on cytokine levels in BAL fluids), the MANS peptide reduced the mean levels of BAL cytokines from 50 to 64%, as illustrated in Fig. 2.

MANS peptide inhibits airway Muc5ac mucin secretion. Mucus cell metaplasia develops preferentially in the larger airways by day 11 in the NE bronchitis model (21), and our present results are consistent with the earlier report. Histological examination of AB/PAS staining of large airways illustrates the development on day 11 of mucus cell metaplasia in mice treated with elastase (Fig. 3). In addition, in the elastase-treatment groups; #differences between groups were significant at P < 0.05. Avg, average.
treated mice, in vivo exposure to methacholine aerosol stimulated release of Muc5ac mucin onto airway surfaces. Mucin secretion was confirmed by ELISA analysis of BAL fluids that were collected after methacholine stimulation and demonstrated a significant increase in the level of Muc5ac mucin protein (Fig. 4). Examination of histological images of the airway confirmed that methacholine stimulation decreased AB/PAS staining of epithelial goblet cells (Fig. 3C). Finally, the MANS peptide delivered before methacholine stimulation dramatically inhibited secretion of mucin; for example, using the BAL Muc5ac level of the vehicle + saline group as a baseline (mean of 167.5 ng/ml), and compared with NE + saline (mean of 307.0 ng/ml) and NE + RNS (mean of 289 ng/ml) groups, MANS reduced the level of Muc5ac in the NE + MANS-treated group (mean of 203.7 ng/ml). Thus BAL levels of Muc5ac in the NE + saline group were observed to be increased by 83.3% above baseline, whereas for the NE + MANS group, the level was only 21.6% above baseline. On a comparison basis, Muc5ac levels in the BAL were lowered by more than one-half in elastase mice pretreated with MANS...
compared with elastase mice pretreated with the scrambled RNS peptide or saline (Fig. 4). The reduced levels of Muc5ac mucin protein in the BAL fluid are consistent with AB/PAS epithelial staining and histological images of the airway in which mice given the MANS peptide had increased epithelial staining compared with RNS- or saline-treated mice (Fig. 3, B, D, and E). A summary of AB/PAS histological scoring results is given in Fig. 5. No evidence of AB/PAS staining was observed in the vehicle- or vehicle/H11001 saline-treated mice (Fig. 3, A and B). NE-conditioned mice that were treated with the MANS peptide exhibited the highest degree of AB/PAS staining (see finding) consistent with the MANS peptide inhibiting methacholine-stimulated mucin secretion.

Fig. 3. Effect of NE treatment on Alcian blue/periodic acid-Schiff (AB/PAS) glycoprotein staining of airway epithelium. Representative histological tissue images of mice airways from: A, Vehicle [1:1 sodium acetate (NaOAc)-to-glycerol] treated; B, Vehicle + saline-treated; C, NE + saline-treated; D, NE + MANS peptide-treated; and E, NE + RNS-treated. Airway image of NE + MANS-treated mouse (D) demonstrates enhanced epithelial staining with AB/PAS consistent with retention of mucin glycoproteins within epithelial goblet cells. Scale bar = 50 μm.

Fig. 4. Influence of methacholine stimulation on Muc5ac mucin secretion. Muc5ac mucin protein level in BAL fluids collected after in vivo stimulation with methacholine aerosol for Vehicle [1:1 sodium acetate (NaOAc)-to-glycerol] and NE-treated mice (mean level ± SE, expressed as nanograms per milliliter, n = 10–12 mice per group). Each group was exposed to methacholine (60 mM) aerosol for 3 min to stimulate airway mucin secretion (see MATERIALS AND METHODS). BAL samples from all NE-treated mice groups had higher levels of Muc5ac than Vehicle-treated controls. #Levels exceeded those of Vehicle controls, and differences were significant at P < 0.05; *Muc5ac was decreased compared with NE + saline and NE + RNS groups, and differences were significant at P < 0.05.

Fig. 5. MANS peptide inhibits mucin glycoprotein secretion from airway epithelium. Histogram summary of AB/PAS scoring of large airways (mean index score ± SE for 8–10 mice per group); representative staining of airways are illustrated in Fig. 3. No evidence of staining with AB/PAS was apparent in airway tissue images sampled from mice treated with Vehicle [1:1 sodium acetate (NaOAc)-to-glycerol] or Vehicle + saline.
MANS peptide inhibits airway mucin secretion

In influence of treatment with MANS peptide on serotonin-induced airway hyperresponsiveness. Responsiveness to serotonin challenge in the elastase- and vehicle-treated mice is illustrated in Fig. 6. By experimental design, each group of mice had been exposed to an aerosol of methacholine on day 11 to induce airway mucin secretion before assessment of airway reactivity to serotonin challenge. In replicate experiments, the elastase-treated mice were found to have no obvious changes in baseline (preserotonin challenge) level of airway resistance; however, the airways became dramatically hyperreactive to cumulative challenge doses of serotonin, as shown by the stepwise enhancement of airflow resistance above baseline as serotonin doses increased from 10 to 100 mg/ml. Interestingly, when NE-treated mice were acutely pretreated with the MANS peptide 48–50 min before serotonin challenge, the enhanced airway responsiveness to serotonin was significantly attenuated. As shown in Fig. 6, treatment with the MANS peptide caused the serotonin responsiveness at each successive dose of serotonin to be dramatically reduced with little or no effect on lung resistance even at the highest serotonin challenge dose of 100 mg/ml. Thus, in day 11 in the NE-induced model of bronchitis, airway reactivity to serotonin develops, and this airway hyperreactivity in response to cumulative aerosol challenge doses with serotonin is acutely and significantly inhibited by pretreatment with the MANS peptide. Compared with MANS, RNS treatment had no influence on altering serotonin-induced hyperreactivity in elastase-treated mice and no effect on responsiveness to cumulative doses of serotonin in vehicle-treated mice.

DISCUSSION

Our results suggest that the MANS peptide, a MARCKS-specific inhibitor, can attenuate bronchial mucus secretion, a common pathological phenotype associated with chronic bronchitis. We established strong evidence in our NE-induced bronchitic mouse model that with delivery of the inhibitory MANS peptide directly to airway surfaces, exocytosis of mucin granules from goblet cells is acutely inhibited with an immediate blockade of agonist-stimulated mucin secretion. Histological images of stained airway epithelial sections demonstrated fully glycoprotein-enriched, AB/PAS-stained goblet cells and correlated with biochemical measures demonstrating reduced levels of Muc5ac mucin protein in BAL fluid.

The effectiveness of the MANS peptide to inhibit mucin secretion in vivo in our model of mucus hypersecretion is completely consistent with previous in vitro and in vivo investigations of mucin secretion. We have demonstrated direct involvement of MARCKS in mucin secretion by NHBE cells in vitro (10) and in mouse airways in vivo (17) whereby stimulation of mucin secretion was attenuated by MANS. Inhibition of mucin secretion by the MANS peptide correlated with the capability of the MANS peptide to attenuate binding of MARCKS to mucin granule membranes and by inference indicated that MARCKS binding to granule membranes is a key regulatory component of mucin secretion.

The present results also established a novel observation that in the NE-induced bronchitic model, airway hyperresponsiveness shown to be present in response to serotonin inhalation develops concurrently with goblet cell metaplasia of the airway epithelium. Immediately following the delivery of agonist stimulus and methacholine to stimulate and induce secretion of mucin, the baseline levels of airflow resistance post-stimulation between elastase- and vehicle-treated mice were equivalent regardless of whether the airway was normal or remodeled (i.e., goblet cell metaplasia). Thus it is not clear...
whether mucus cell metaplasia of the epithelium per se contributes significantly to airflow resistance in this model. However, in remodeled mucus cell metaplastic airways, pretreatment of the airway directly with the MANS peptide before stimulation with the mucus secretagogue, methacholine, attenuated airway hyperresponsiveness in response to cumulative aerosol challenge with serotonin. Serotonin has been used previously in mice models to demonstrate responsiveness of the bronchial musculature and to accentuate differences in airway responsiveness between inbred mouse strains (7, 12). To our knowledge, serotonin has not been shown to influence mucus secretion, and thus the MANS peptide-related attenuation of airway hyperresponsiveness would seemingly be associated with direct effects on airway smooth muscle contraction, indirect effects on baseline airway lumens related to inhibition of mucus secretion, or indirect effects of secreted mucins on airway reactivity.

In models in which the airway has been remodeled with mucus metaplasia, an additional factor to be considered with respect to airway narrowing and functional response to agonist stimulation is that excessive secretion of mucus onto airway epithelial surfaces and/or delays in mucus clearance may influence airway reactivity. For example, in a dog model of mucus hypersecretion, when secretion levels were elevated, the dogs were relatively unresponsive to an aerosolized agonist opposed to when the agonist was delivered by a vascular route (9). These results were suggestive of delay or restriction of the agonist in gaining access to the target site. Although, in our model, mucus-related effects seem potentially possible as serotonin was delivered by a respiratory route, there was no obvious diminution in serotonin sensitivity, and lung airflow resistance changes were similar in intensity to observations we have made in other mouse models having normal epithelial histology (unpublished observations). When MANS peptide was added as a pretreatment and hyperresponsiveness to serotonin became blunted, this influence could not have been related to agonist/target accessibility, since mucus secretion was demonstrated to be inhibited (as shown with BAL mucin protein levels and supported by the histological AB/PAS scoring index). In addition, the ability of the MANS peptide to effectively blunt airway hyperresponsiveness had a duration of at least 45–50 min postdelivery.

Finally, after pretreatment with the MANS peptide, the profile of proinflammatory cytokines in BAL fluids (and indicative of lung inflammation) was significantly decreased in the bronchiectasis model. Parallel to the influence of MANS peptide on agonist-stimulated secretion of mucus and a reduction in BAL levels of Muc5ac mucin, the levels of the investigated proinflammatory cytokines, IL-8, IL-1β, IL-6, and TNFα, were also influenced by the MANS peptide with reductions that ranged between 50 and 60%, compared with mice pretreated with the scrambled RNS peptide. These findings are similar to results we (8) described for a pulmonary injury model that was induced by preexposure to ozone. In that study, the MANS peptide, administered as airway prophyaxis before ozone challenge, practically abolished BAL levels of inflammatory cytokines, IL-6 and KC, with reductions ranging between 82 and 95%, respectively.

In previous studies, we have shown that the MANS peptide has, in addition to its attenuating effect on mucin secretion, two potential anti-inflammatory effects. First, it can inhibit migration of neutrophils in vitro (5), and, second, it can inhibit release of inflammatory mediators from isolated leukocytes (19). Thus a potential mechanism whereby MANS may decrease inflammation in vivo can relate to both of these effects: it can block both influx of inflammatory cells into the lung by attenuating their migration toward a chemotactic stimulus and also inhibit release of mediators from these cells. The data reported here show that both cell numbers and inflammatory mediator and cytokine levels in BAL were decreased after administration of the MANS but not RNS peptide, so it is entirely conceivable that both of these mechanisms came into play in the treated animals, even in this relatively short time period and after exposure of the animals to the peptides via intratracheal instillation. Clearly, additional studies relating to the role of MARCKS protein, as well as the MANS peptide, in affecting inflammation in vivo are called for, as the above studies relate to work done on isolated leukocytes.

In summary, the MANS peptide, a specific inhibitor of MARCKS protein, administered directly to the airway, inhibits agonist-induced mucus secretion in a bronchitic model of mucus cell metaplasia. Also, the MANS peptide affords protection from NE-related airway inflammation and limits agonist-induced narrowing of airway lumens, two clinical phenotypes commonly associated with abnormal airway function in chronic bronchitis. As additional information evolves on the pharmacokinetics of the MANS peptide, this or similar peptides may be helpful for relief of abnormal phenotypes and inflammation associated with obstructive-type airway disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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