fluid of patients with acute lung injury. Am J Respir Crit Care Med 2001; 163:166–172
35 Matthias MA, Wiener-Kronish JP. Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. Am Rev Respir Dis 1990; 142:1250–1257
36 Ware LB, Matthay MA. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. Am J Respir Crit Care Med 2001; 163:1376–1383
37 Nielsen VG, Baird MS, Chen L, et al. DETANONOate, a nitric oxide donor, decreases and/or nitric-sensitive alveolar fluid clearance in rabbits. Am J Respir Crit Care Med 2000; 161:1154–1160
40 Beckman JS, Beckman TW, Chen J, et al. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A 1996; 93:1620–1624
50 Zhu S, Haddad IY, Matalon S. Nitration of surfactant protein A (SP-A) tyrosine residues results in decreased mannose binding ability. Arch Biochem Biophys 1996; 333:282–290

Goblet Cell and Mucin Gene Abnormalities in Asthma*

John V. Falot, MD

Goblet cell hyperplasia (GCH) has been established as a pathologic characteristic of mild, moderate, and severe asthma. Abnormalities in goblet cell number are accomplished by changes in stored and secreted mucin (MUC). The functional consequences of these changes in MUC stores and secretion can contribute to the pathophysiologic mechanisms for multiple clinical abnormalities in patients with asthma, including sputum production, airway narrowing, exacerbations, and accelerated loss in lung function. CD4+ T cells and their T-helper type-2 cytokine products are important mediators of GCH, and MUC5AC is the dominant MUC gene that is expressed in goblet cells. The mechanism of cytokine-induced GCH, the relationships between MUC gene up-regulation and GCH, and the role of ion channels are all currently being explored. The process of working out the molecular mechanisms of GCH and goblet cell degranulation should provide new targets for novel therapeutic interventions. Such new treatments are urgently needed, because mucous hypersecretion is an important cause of morbidity and mortality in patients with asthma, and no specific treatments are available.

(CHEST 2002; 122:3205–3206)

Key words: asthma; goblet cells; mucin genes; mucin glycoproteins

Abbreviations: AB = alcian blue; EGFr = epidermal growth factor receptor; GCH = goblet cell hyperplasia; IL = interleukin

*From the Division of Pulmonary and Critical Care Medicine, and the Cardiovascular Research Institute, University of California at San Francisco, San Francisco, CA. This research was supported by grants R01 HL61662 and P50 HL56385 from the National Heart, Lung, and Blood Institute. Correspondence to: John V. Falot, MD, Box 0111, University of California, San Francisco, 505 Parnassus Ave, San Francisco, CA 94143; e-mail: jfalot@tsa.ucsf.edu

Remodeling and Repair in Respiratory Diseases

3205

Downloaded From: http://journal.publications.chestnet.org/pdaccess.asmx?url=/data/journals/chest/21986/ on 06/26/2017
The cellular sources of mucin (MUC) glycoproteins (termed mucins) in the airway are goblet cells in the surface epithelium and mucus cells in submucosal glands. Mucins are the major constituents of airway mucus and contribute significantly to its viscoelastic and adhesive properties. Other important constituents of airway mucus include plasma-derived proteins, especially albumin, and products of cell death such as DNA and actin. Mucus hypersecretion is therefore a complex pathologic process that involves the mechanisms that are responsible for hyperplasia and degranulation of mucus-secreting cells, microvascular remodeling and leakage, and chemoattraction of inflammatory cells.

Mucins are important components of host defense, but they represent an important cause of airway obstruction when secreted in excess. Fatal asthma, for example, is nearly always associated with airway occlusion from mucous plugs. In addition, sputum production is a common symptom in patients with asthma, especially during asthma exacerbations. Furthermore, a history of sputum production is independently associated with an accelerated rate of decline in FEV₁, suggesting that mucus hypersecretion is therefore a complex pathologic process of inflammatory cells.

Whether goblet cells or submucosal gland cells are the principal sources of mucins in airway secretions in asthma or in other airway diseases is not known. In disease states, it is likely that the relative contribution of goblet cells and gland cells varies according to airway level, disease severity, and disease type. In asthma patients, it is clear that goblet cells contribute mucins to airway secretions, at least in case of fatal asthma in which luminal mucins are contiguous with intracellular mucins in goblet cells. Submucosal glands are also prominent in cases of fatal asthma in which the gland ducts often become ectatic (termed bronchial diverticulitis).

Over the past 10 years, animal models of allergic airway disease have shown that CD4⁺ T cells mediate allergen-induced goblet cell hyperplasia (GCH). In addition, detailed transgenic and gene deletion studies in experimental mice have shown that T helper type 2 (Th2) cytokines are important growth factors for goblet cells. Furthermore, knowledge of the signaling pathways that lead from Th2 cytokine stimulation to MUC gene up-regulation in goblet cells has advanced considerably.

In this review, I will summarize the MUC gene and goblet cell abnormalities that occur in patients with clinical asthma, and I will briefly review the mechanisms of GCH and degranulation that may be especially relevant in case of asthma.

MUC Gene Abnormalities in Asthma

Mucins are large complex molecules consisting of a peptide backbone and numerous oligosaccharide side chains that represent the products of MUC genes and glycosyltransferase genes, respectively. A total of 12 human MUC genes have been identified and numbered in chronologic order of their description, as follows: MUC1–4, MUC5AC, MUC5B, MUC6–8, and MUC11–13. Of these, the complete complementary DNA sequences for only six mucins (MUC1, MUC2, MUC4, MUC5B, MUC5AC, and MUC7) have published. The genes for MUC2, MUC5AC, MUC5B, and MUC6 are located on chromosome 11p15 and code for the major gel-forming secreted mucins. This is in contrast to the genes for MUC1, MUC3, MUC4, MUC12, and MUC13, which code for nonsecreted transmembrane MUC molecules.

Using real-time reverse transcriptase polymerase chain reaction, we recently examined the expression levels of MUC genes in homogenates of bronchial biopsy specimens obtained from asthmatic subjects and healthy control subjects. The most frequently expressed MUC gene in the airway biopsy specimens from both groups was MUC5AC (Fig 1). In addition, MUC5AC expression was approximately 60% higher than normal in the asthmatic subjects. This increase was not statistically significant, but it was consistent with increased immunohistochemical expression of MUC5AC in bronchial biopsy specimens from the same subjects. Although much lower than MUC5AC, detectable gene expression was also found for MUC1, MUC2, MUC4, MUC5B, and MUC7 in the biopsy specimens from the asthmatic subjects. The expression levels of MUC2 and MUC4 were significantly increased in the asthmatic subjects, whereas the expression of MUC5B was significantly decreased. The significance of these data are uncertain, because little is known about the physical properties of the MUC products of different MUC genes and about the specific effects on mucus rheology when changes in MUC gene expression occur in disease. Also unknown are the concentration levels of the secreted products of different MUC genes in the airway secretions of asthma patients. The available data from healthy control subjects, and from patients with chronic bronchitis and cystic fibrosis, show that MUC5AC and MUC5B are the principal secreted mucins in airway secretions. Although MUC2 protein expression in the airway has been found immunohistochemically in some studies, it has not been found consistently.

Goblet Cell Abnormalities in Clinical Asthma

Sequential alcian blue (AB) and periodic acid-Schiff (PAS) staining of airway tissue sections allows clear visualization of mucins in secretory cells (Fig 2). The availability of these stains has facilitated pathologic studies of goblet cells in airway tissue from humans and experimental animals. Despite this, and despite the accessibility of the airway epithelium to sampling by bronchoscopy, most pathologic studies of goblet cells in human asthma have been in postmortem cases of fatal asthma. Here, GCH is a consistent finding. Clear less is whether GCH is a characteristic of less severe forms of asthma. One study of bronchial biopsy specimens from subjects with mild asthma found no increase in airway epithelial goblet cells, but this study may have underestimated goblet cell numbers because it relied on hematoxylin-eosin staining of the epithelium and on semi-quantitative methodology to
count goblet cells. My laboratory recently analyzed goblet cell size and number in bronchial biopsy specimens from asthmatic subjects with mild and moderate disease. AB/PAS staining was used to identify stored mucins in goblet cells, and methods of design-based stereology were applied to quantify goblet cell size and number.

Stereology provides quantitative three-dimensional information from two-dimensional observations in tissue, and "design-based" stereology indicates that the measurement protocol is designed to ensure that the sampled fields of tissue are representative of the whole tissue (i.e., that they are unbiased). Fundamental stereologic equations allow the estimation of volume, surface area, length, and numbers of microscopic objects from two-dimensional images. In designing a measurement protocol for goblet cells, it is important for accuracy to analyze as many

**Figure 1.** The expression of nine different MUC genes in homogenates of endobronchial biopsy specimens from 8 healthy subjects and 11 asthmatic subjects is shown. Gene expression was measured by real-time reverse transcriptase polymerase chain reaction, and the expression of MUC genes is represented as the ratio of MUC gene-specific messenger RNA copy numbers to those of the transferrin receptor (housekeeping gene). Data are expressed as the mean ± SD. * = messenger RNA expression in asthmatic subjects is significantly greater than that in healthy subjects (p < 0.005). Reproduced with permission from Ordóñez et al.15

**Figure 2.** Photomicrograph of 3-μM sections of bronchial biopsy specimens from an asthmatic subject before and after aerosolized allergen challenge. The stain used was sequential AB/PAS. **Left,** A: a representative biopsy specimen section in the baseline state. **Middle,** B: a representative biopsy specimen section 1 h postchallenge. **Right,** C: a representative biopsy specimen section 24 h postchallenge. Allergen challenge does not show a reduction in AB/PAS-stained mucins in the epithelial goblet cells. In fact, stained mucins tend to increase in the epithelium at the 24-h time point. Reproduced with permission from Hays et al.49

Downloaded From: http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21986/ on 06/26/2017
different biopsy specimens from each subject as possible. Therefore, measurements were made in multiple high-power fields from as many as seven biopsy specimens per subject (total, > 100 fields per subject) using an integrated measurement system, which included a microscope, automated microscope stage, video camera, and computer equipped with stereology software. The cell numbers measured using stereology represent true numeric densities and are preferable to cell profile counting, which is subject to a cell size bias (ie, the overestimation of larger cells and the underestimation of smaller cells).

Goblet cell numbers were 2.5-fold higher than normal in the airway epithelium in subjects with asthma. This resulted in a volume of stored MUC in the epithelium that was approximately three times higher than normal (Fig 3). Also, higher stored MUC levels in the epithelium were associated with lower levels of secreted MUC in samples of induced sputum, and vice versa. Because secreted MUC levels were higher in patients with lower FEV1 values, it is possible that the pathogenesis of airway obstruction in more severe forms of asthma involves ongoing MUC hyperscretion from goblet cells. In addition, the higher levels of stored mucins in goblet cells in patients with mild asthma suggest a mechanism for mucus formation and airway obstruction in these patients during acute exacerbations.

MECHANISMS OF GCH IN ASTHMA

Knowledge of the growth factors driving GCH or metaplasia in allergic airway diseases has advanced greatly due to experiments in animal models. Airway allergen challenge in sensitized animals causes marked GCH and the major effector cell is the Th2 subtype of the CD4+ T cell. The evidence for this is compelling. The adoptive transfer of Th2 cells, but not Th1 cells, into the lungs of naive mice causes GCH and the overexpression of Th2 cytokines in mice, including interleukin (IL)-4, IL-5, IL-9, and IL-13, leads to GCH. In contrast, there does not appear to be a role for eosinophils in mediating allergen-induced or Th2 cytokine-induced GCH, because several studies have shown that allergen-induced or Th2 cytokine-induced eosinophilia and mucus cell hyperplasia are dissociated.

The mechanism by which Th2 cytokines induce GCH in the airway is uncertain. Several epithelial cells are candidate precursor cells for goblet cells, including basal cells and Clara cells. Airway MUC5AC expression is increased in the airway epithelium in animal models of allergic airway disease as well as in human asthma, and it is possible that up-regulation of MUC5AC in these cells is the critical first event leading to cell differentiation to goblet cells. However, only IL-9, and not IL-4 or IL-13, increases MUC gene expression in human airway epithelial cell culture systems. Alternatively, therefore, MUC5AC expression may simply represent a cellular marker of goblet cells hyperplasia induced by Th2 activation of other intracellular signaling mechanisms. Investigations in this area recently have turned up some surprising findings. For example, recently it has been found that a calcium-activated chloride channel (gob-5 in the mouse and hCLCA1 in humans) is induced by Th2 cytokine stimulation of epithelial cells. In addition, the overexpression of this channel in cultured airway epithelial cells induces mucus cell metaplasia. Interestingly, another ion channel, a sodium potassium chloride cotransporter (NKCC1), is up-regulated in goblet cells in cases of human asthma. Thus, the overexpression of ion channels may be an important downstream effect of Th2 cytokine stimulation of epithelial cells. The mechanism by which the activation of these channels leads to GCH in asthma patients is unknown.

An important role for the epidermal growth factor receptor (EGFr) and its ligands in GCH in asthma patients is also evident from recent studies. For example, selective inhibitors of EGFr tyrosine kinase block IL-13-induced MUC gene expression in rat airways and epithelial cell proliferation in cultured bronchial epithelial cells. The critical ligand for the EGFr in these instances is transforming growth factor-α, which is released from neutrophils or from the airway epithelium itself.

In summary, it is clear that the mechanisms of allergen-induced GCH involve CD4+ T cells and Th2 cytokines (Fig 4). However, much remains to be learned about the mechanisms of cytokine-induced GCH, the interaction of Th2 cytokines and the EGFr system, and the role of ion channels in goblet cell differentiation.

GOBLET CELL DEGRANULATION

The principal functional consequence of increased goblet cell numbers is thought to be hypersecretion of MUC glycoproteins when goblet cells degranulate. The mechanisms of goblet cell degranulation in vivo are separate from those of GCH or metaplasia. MUC molecules are...
stored in MUC granules in the cytoplasmic compartment of goblet cells. The packaging of the large MUC molecules within the storage granules requires condensation, which is a process that is made difficult by the polyanionic nature of mucins. High concentrations of calcium in the granules function as shielding cations to nullify the repulsive forces within the MUC molecule. The outer surface of the granule fuses with the inner surface of the cell membrane during MUC secretion and forms a channel through which intracellular calcium exits and extracellular water enters the granule. The loss of calcium allows electrostatic repulsion to rapidly expand the MUC macromolecule, which is simultaneously...

**Figure 4.** Simplified schematic showing possible mechanisms of GCH in asthma patients. Th2 cytokines (e.g., IL-9 and IL-13) secreted by CD4+ T cells cause increased MUC5AC gene expression, which may be a critical signal for goblet cell differentiation from basal cells or Clara cells. The effect of Th2 cytokines on MUC5AC in *vivo* may be mediated by a variety of other molecules, including the EGFR and its ligand transforming growth factor-α (TGFα), and by ion channels such as the calcium-activated chloride channel (hCACL1) or the sodium potassium chloride cotransporter (NKCC1).

**Figure 5.** Left: the number of eosinophils in bronchial biopsy specimens at baseline and at two time points after allergen challenge. The number of eosinophils per volume of biopsy (Nv eos, bio) increased significantly at 1 h and remained higher than baseline at 24 h. Right: stored MUC in the airway epithelium at baseline and at two time points after allergen challenge. The volume of stored MUC per volume of epithelium (Vv muc, epi) did not decrease at the 1-h or 24-h time point, as would have been expected if goblet cells had degranulated. Instead, there was a tendency for the volume of MUC in the epithelium to increase at 24 h. The data are expressed as the mean ± SD. * = significantly greater than baseline (p < 0.05). Modified with permission from Hays et al.49
hydrated with incoming water. The large hydrated macromolecule then erupts from the cell like a "jack-in-the-box." Through this secretion mechanism, secreted MUC macromolecules are much greater in volume than the volume of stored mucus in the goblet cell granules.

In rodents, goblet cell degranulation and mucus occlusion of the airways has occurred following high-dose allergen challenge in sensitized animals. A specific 5-lipoxygenase inhibitor (zileuton) prevents degranulation, indicating that leukotrienes mediate this effect in this model. Other possible inflammatory mediators of allergen-induced goblet cell degranulation include chymase, eosinophil cationic protein, and neutrophil elastase.

The effect of allergen challenge on goblet cell degranulation in vivo in human subjects has recently been examined. Eight allergic asthmatic subjects were challenged with an aerosol of aeroallergen using a whole-lung challenge protocol. Although eosinophilic inflammation occurred as soon as 1 h after the challenge, there was no evidence of goblet cell degranulation in the airway biopsy specimens or in lavage samples at time points of either 1 h or 24 h after the challenge (Fig 2, S). This suggests that the mechanisms of degranulation in human asthma are complex. Important inhibitory mechanisms must be in place in human asthma that prevent the degranulation of goblet cells even on allergen exposure, except in specific clinical circumstances. An example of such a circumstance might be allergen exposure that is coincident with a viral infection of the airway.

Our knowledge of the mechanisms linking cell surface stimulation with a MUC secretagogue and the extracellular release of MUC granule contents is quite rudimentary, but recent advances have been made. Specifically, a role has been discovered for myristolylated alanine-rich C kinase substrate (MARCKS) protein, whereby secretagogue-induced activation of protein kinase C phosphorylates MARCKS, causing translocation of MARCKS from the plasma membrane to the cytoplasm. In the cytoplasm, MARCKS is dephosphorylated, associates with both actin and myosin, and also interacts with MUC granule membranes. In this way, MUC granules are linked to the contractile cytoskeleton facilitating their movement to the cell periphery and subsequent exocytosis.

As more is learned about the specific mechanisms of degranulation, it should be possible to develop specific inhibitors of degranulation. Currently, no such inhibitors exist, and their development would open up a whole new treatment strategy for mucus hypersecretion in patients with asthma and other airway diseases.

REFERENCES
3 Huber HC, Koessler KK. The pathology of bronchial asthma. Arch Intern Med 1922; 30:699–760
4 Houston JC, De Navasquez S, Trounce JR. A clinical and pathological study of fatal cases of status asthmaticus. Thorax 1953; 8:207–213
22 Blyth DI, Pedrick MS, Savage TJ, et al. Allergen-induced airway goblet cell hyperplasia (GCH) in mice: suppression of developing and established GCH by dexamethasone (Dex). Am J Respir Crit Care Med 1997; 155:A291
Hypoxic Activation of Adventitial Fibroblasts*

Role in Vascular Remodeling

Kurt R. Steinmark, MD; Evgenia Gerasimovskaya, PhD; Raphael A. Nemenoff, PhD; and Mita Das, PhD

Substantial experimental evidence supports the idea that the fibroblast may play a significant role in the vascular response to injury, especially under hypoxic conditions. Fibroblasts have the ability to rapidly respond to hypoxic stress and to modulate their function to adapt rapidly to local vascular needs. Fibroblasts appear to be uniquely equipped to proliferate, transdifferentiate, and migrate under hypoxic conditions. Proliferative responses to hypoxia depend on the activation of Gαi and Gq kinase family members, and on the subsequent stimulation of protein kinase C and mitogen-activated protein kinase family members. Extracellular nucleotides (eg, adenosine triphosphate [ATP]) are likely to be increased in the hypoxic adventitial compartment and can act as autocrine/paracrine modifiers of the hypoxia-induced proliferative response. The proliferative effects of ATP appear to be mediated largely through G-protein-coupled P2Y receptors in fetal and neonatal fibroblasts. Hypoxia, acting through Gαs-coupled pathways, can also directly up-regulate α-smooth muscle actin expression in fibroblast subpopulations, suggesting that hypoxia may play a

Remodeling and Repair in Respiratory Diseases

326S


Oikawara Y, Lei XF, Stampfl MR, et al. Cytokine and eosi


enate reveals increased expression of a Na+K+-Cl- cotrans

porter (NKCC1) in asthmatic subjects. Genome Res 2001; 11:1473–1483


enate reveals increased expression of a Na+K+-Cl- cotrans

porter (NKCC1) in asthmatic subjects. Genome Res 2001; 11:1473–1483


enate reveals increased expression of a Na+K+-Cl- cotrans

porter (NKCC1) in asthmatic subjects. Genome Res 2001; 11:1473–1483

