Up-Regulation of MUC18 in Airway Epithelial Cells by IL-13

Implications in Bacterial Adherence

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Airway bacterial infections are a major problem in lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis. Increased Th2 cytokines, such as IL-13, are observed in lung diseases and may contribute to bacterial infections. How Th2 cytokines affect bacterial infection remains unknown. MUC18, an adhesion molecule shown to be involved in the pathogenesis of malignant melanoma, has been recently identified in airway epithelial cells of patients with COPD. We investigated MUC18 regulation by IL-13 and the role of MUC18 in bacterial adherence to epithelial cells. Human airway tissues, brushed bronchial epithelial cells from normal subjects and subjects with asthma, and epithelial cell lines (e.g., HEK293 cells) were used to study the regulation of MUC18 by IL-13 and the involvement of MUC18 in bacterial (e.g., *Mycoplasma pneumoniae* [Mp] and nontypeable *Haemophilus influenzae* [NTHi]) adherence to epithelial cells. Asthmatic bronchial epithelium expressed higher levels of MUC18 than normal bronchial epithelium. IL-13 increased MUC18 in cultured bronchial epithelial cells from normal subjects and particularly from subjects with asthma. IL-13-induced MUC18 expression may be modulated in part through transcription factor specificity protein 1. Overexpression of human MUC18 in HEK293 cells increased cell-associated Mp and NTHi levels. Moreover, MUC18 was shown to directly interact with Mp and NTHi. These results for the first time show that an allergic airway milieu (e.g., IL-13) increases MUC18 expression, which may contribute to increased bacterial infection/colonization in asthma and other lung diseases.

Keywords: airway epithelial cells; MUC18; IL-13; bacteria

MUC18, also known as CD146 or melanoma cell adhesion molecule, is a cell membrane glycoprotein belonging to the immunoglobulin superfamily (1). It is controversial whether MUC18 should be included in the mucin (i.e., cell membrane-tethered mucins) family because it lacks the typical tandem repeat structure of mucins. Human MUC18 is a type I transmembrane glycoprotein with 646 amino acids (molecular weight, 113 kD) (2, 3). MUC18 aberrant overexpression was initially identified in human malignant melanoma cells, which may promote tumor metastasis (4–6). Nonmalignant cells, such as brushed bronchial epithelial cells, from patients with COPD have been shown to express higher levels of MUC18 than nonmalignant cells normal subjects (7). However, MUC18 regulation, and especially function in airway epithelial cells, remains poorly understood.

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CLINICAL RELEVANCE

This study for the first time demonstrates the increased expression of MUC18 in asthmatic airway epithelium and the up-regulation of MUC18 by Th2 cytokine IL-13 in cultured primary human brushed bronchial epithelial cells. Moreover, MUC18 was shown to be involved in bacterial adherence to epithelial cells. Our findings provide a novel mechanism underlying increased susceptibility of allergic airways to bacterial infection and an alternative approach to attenuate bacterial infections in asthma and other lung diseases.

Airway epithelial cells serve as the first line of host defense against various environmental insults, such as bacteria. Despite recent advances in therapy, airway bacterial infections are a significant challenge in healthcare of several prominent lung diseases, including asthma and chronic obstructive pulmonary disease (COPD) (8, 9). A subset of patients with asthma presents Th2-biased airway allergic inflammation (10). Some COPD lungs also present a Th2 phenotype (11). Many species of bacteria have been identified in the airways of patients with asthma or COPD (8, 9, 12, 13). However, the role of bacteria in disease acute exacerbations as well as progression remains controversial (14, 15). The common bacteria seen in asthma and COPD airways include, but are not limited to, *Mycoplasma pneumoniae* (Mp), nontypeable *Haemophilus influenzae* (NTHi), and *Moraxella catarrhalis* (12, 13). Our previous studies have shown that the Th2 cytokine IL-13 increased Mp load in well differentiated human airway epithelial cells under the air-liquid interface (ALI) cultures (16, 17), suggesting a damaging role of IL-13 in epithelial defense against pathogenic bacteria. However, the mechanisms by which IL-13 increases bacterial load on airway epithelium remain largely unknown.

Our current study explored the novel mechanisms underlying increased bacterial load in IL-13-exposed human airway epithelial cells. We hypothesized that Th2 cytokines increase airway epithelial MUC18 expression, which functions as an adhesion molecule to retain the bacteria (e.g., Mp and NTHi) on epithelial cells. To test our hypothesis, we used human airway tissues, primary brushed bronchial epithelial cells from normal subjects and subjects with asthma, and epithelial cell lines (e.g., HEK293 cells) to investigate the impact of IL-13 on MUC18 expression and to study the involvement of MUC18 in bacterial adherence to epithelial cells.

MATERIALS AND METHODS

Study Participants, Bronchoscopy, and Bronchial Epithelial Cell Processing

Bronchoscopy with endobronchial epithelial brushings was performed on 11 human subjects (normal, $n = 4$; asthma, $n = 7$). The clinical

TABLE 1. CHARACTERISTICS OF HUMAN STUDY SUBJECTS

	Age	Sex (M/F)	Smoking (<i>pack-years</i>)	FEV ₁ (% <i>predicted</i>)	FEV ₁ /FVC%	Medications
Normal (<i>n</i> = 4)	58.0 ± 4.1	2/2	0 (<i>n</i> = 2) 22.0 ± 12.8 (<i>n</i> = 2)	91.7 ± 8.6	83.7 ± 1.5	None
Asthma (<i>n</i> = 7)	48.0 ± 4.7	3/4	0 (<i>n</i> = 4) 7.4 ± 4.2 (<i>n</i> = 3)	87.3 ± 9.0	70.7 ± 5.0	ICS: 6/7 β ₂ agonist: 6/7

Definition of abbreviation: ICS = inhaled corticosteroids.

characteristics for all subjects are shown in Table 1. All subjects with asthma met the American Thoracic Society criteria for mild to moderate asthma (18). Bronchial brushings were performed as previously described (17, 19). Up to six brushings were obtained per subject for MUC18 protein and mRNA measurements and cell culture. Our research protocols were approved by the institutional review board at National Jewish Health, and all subjects provided written informed consent.

Brushed Bronchial Epithelial Cell ALI Cultures

Brushed bronchial epithelial cells were cultured under ALI conditions with or without IL-13 (10 ng/ml) treatments as previously reported (17, 19–22) (*see details in the online supplement*). After 16 days of treatment, cells were harvested into TRIzol (Invitrogen, Carlsbad, CA) and Western lysis buffer for MUC18 mRNA and protein detection, respectively.

MUC18 Immunohistochemistry

MUC18 protein was evaluated in airway tissues of three normal donors and one subject with fatal asthma as well as in cytospin slides of brushed bronchial epithelial cells from normal subjects and subjects with asthma (*see details in the online supplement*).

RNA Interference of Transcription Factor Specificity Protein 1 in NCI-H292 Cells

To determine if specificity protein 1 (Sp1) is involved in IL-13–induced MUC18 expression, RNA interference was used to knockdown Sp1 in IL-13–treated NCI-H292 cells, a human pulmonary mucoepidermoid carcinoma cell line (ATCC, Manassas, VA), because transfection of

siRNA in ALI cultures of primary human airway epithelial cells is difficult. Briefly, NCI-H292 cells were transfected using a control siRNA or Sp1 siRNA (Santa Cruz Inc., Santa Cruz, CA) following the manufacturer's instructions. The cells were then stimulated with IL-13 (10 ng/ml) or cell culture medium (control) for 48 hours to measure MUC18 and Sp1.

Generation of HEK293 Cells Stably Overexpressing Human MUC18 Protein

To study the role of MUC18 in bacterial adherence to epithelial cells, HEK293 cells (ATCC, Manassas, VA) that stably overexpress human MUC18 protein were generated (*see details in the online supplement*). MUC18 protein expression in HEK293 cells was verified by Western blot.

Bacterial Adherence to HEK293 Cells that Stably Overexpress Human MUC18 Protein

Mp (strain FH, ATCC 15531) and NTHi 12 were prepared as previously reported (23, 24).

Bacterial adherence assay was performed by inoculating Mp or NTHi to HEK293 cells with or without MUC18 overexpression. Cell-associated bacteria were then quantified.

To further confirm the involvement of MUC18 in NTHi adherence, HEK293 cells with or without MUC18 overexpression were pretreated with a fully human anti-MUC18 antibody ABX-MA1 (10 μg/ml) (Amgen, Inc., San Diego, CA) (25) or human IgG (control, 10 μg/ml) (R&D Systems, Minneapolis, MN) for 2 hours followed by NTHi infection (10 CFU/cell). After 24 hours, nonadherent bacteria were washed three times with PBS, and cell-associated NTHi was quantified.

Figure 1. MUC18 protein immunostaining in human lung tissues and brushed bronchial epithelial cells. (A) Lung tissues from a subject with fatal asthma and a subject without lung disease (normal control) were immunostained using a rabbit anti-human MUC18 antibody (Epitomics, Inc., Burlingame, CA). As compared with the normal airway, fatal asthmatic airway epithelium demonstrated increased MUC18 protein staining, predominantly at the apical surface. No staining was seen in the same fatal asthmatic airway tissue incubated with a rabbit IgG (negative control). Original magnification, ×200. (B) Brushed noncultured bronchial epithelial cells on cytospin slides were immunostained with the same anti-MUC18 antibody as described for the lung tissue. The percentage of MUC18(+) cells in subjects with mild to moderate asthma (*n* = 7) was significantly higher than that in normal subjects (*n* = 4). The horizontal bars represent medians. Original magnification, ×400.

Figure 2. Effects of IL-13 on MUC18 expression of cultured bronchial epithelial cells obtained through bronchial brushings in normal subjects ($n = 4$) and subjects with mild to moderate asthma ($n = 7$). Brushed bronchial epithelial cells were cultured under the air-liquid interface conditions for 16 days in the presence or absence of IL-13 (10 ng/ml) for MUC18 mRNA and protein measurements using real-time quantitative RT-PCR and Western blotting, respectively. Although IL-13 increased MUC18 mRNA levels in normal subjects and in subjects with asthma (A), it only significantly increased MUC18 protein (B and C) in subjects with asthma. Data are expressed as means $\pm$ SEM.

To determine the direct interaction of bacteria with MUC18 protein, a direct binding assay of recombinant human full-length MUC18 protein with Mp or NTHi was performed as described in the online supplement.

Figure 3. IL-13 increases levels of *Mycoplasma pneumoniae* (Mp) associated with NCI-H292 cells. Cells were treated with or without IL-13 (10 ng/ml) for 48 hours, followed by Mp infection (10 CFU/cell) for 24 (A) or 48 (B) hours. After three washes with PBS, cell-associated Mp levels were quantified. $n = 4$ replicates.

Western Blot Analysis

Western blot was used to examine epithelial MUC18 and Sp1 proteins. Densitometry was performed to quantify the protein in relation to β -actin or GAPDH (see details in the online supplement).

Real-Time Quantitative RT-PCR

MUC18 mRNA in epithelial cells was determined by RT-PCR and real-time quantitative PCR and analyzed using the comparative threshold cycle method by normalizing to the housekeeping gene GAPDH (26) (see details in the online supplement).

Statistical Analysis

For normally distributed data, one-way ANOVA was used for multiple comparisons, and a Tukey's *post hoc* test was applied where appropriate. Student's *t* test was used when only two groups were compared. Nonnormally distributed data were compared using the Wilcoxon rank-sum test. $P \leq 0.05$ was considered significant.

RESULTS

Asthmatic Airway Epithelial Cells Express MUC18

Immunohistochemistry was performed to localize MUC18 protein in airway epithelium in three normal donor lungs and in a fatal asthmatic lung that presented a typical Th2 phenotype, including goblet cell metaplasia, eosinophil infiltration, and thickening of subepithelial basement membrane. Although the normal airway epithelium demonstrated minimal MUC18 protein staining, the airway epithelium from the subject with fatal asthma showed strong MUC18 protein staining, particularly at the apical surface (Figure 1A). Basal cells also expressed MUC18 (see Figure E1 in the online supplement). MUC18 colocalization suggested ciliated cells as the primary MUC18-expressing cells in airway epithelium (Figure E2). The MUC18 mRNA level in whole lung from the subject with fatal asthma, as determined by Affymetrix microarray, was 3.2-fold higher than that in the normal lung ($n = 1$).

To further confirm up-regulation of MUC18 in asthmatic airway epithelial cells, immunostaining was performed on cytospin slides of brushed (noncultured) bronchial epithelial cells from four normal subjects and seven patients with mild to moderate asthma. The clinical characteristics of human subjects are presented in Table 1. The percentage of MUC18 (+) bronchial epithelial cells was significantly higher in subjects with asthma than that in normal subjects (Figure 1B). Furthermore, MUC18 mRNA was measured in brushed bronchial epithelial cells from four normal subjects and three subjects with asthma from whom there were cells left for quantitative PCR after cells were allocated for cell culture experiments. MUC18 mRNA levels in bronchial epithelial cells of subjects with asthma were also higher than those in normal subjects (MUC18 mRNA relative levels: asthma = 4.4 ± 0.16 versus normal = 2.5 ± 0.3 ; $P = 0.034$).

Effects of IL-13 on MUC18 Expression in Cultured Brushed Human Bronchial Epithelial Cells

The impact of IL-13 on MUC18 expression was examined in cultured brushed bronchial epithelial cells from four normal subjects and seven subjects with mild to moderate asthma under the ALI cultures. IL-13 significantly increased MUC18 mRNA levels in normal subjects and in subjects with asthma (Figure 2A). Although MUC18 mRNA levels were similar ($P > 0.05$) in nonstimulated (–) cells from both subject groups, the induction of MUC18 mRNA by IL-13 in normal subjects was greater than that in subjects with asthma. Unlike MUC18 mRNA data, IL-13 only significantly increased MUC18 protein expression in asthmatic bronchial epithelial cells (Figures 2B and 2C).

Mechanisms of IL-13-Induced MUC18 Expression in Epithelial Cells

To reveal the mechanisms by which MUC18 is up-regulated by IL-13, we used NCI-H292 cells to knockdown Sp1, one of the transcription factors that has been identified in the promoter of MUC18 in melanoma cells (1).

Using Mp as an infection model, NCI-H292 cells treated with IL-13 demonstrated higher Mp levels than those without IL-13 treatment (Figure 3), which is similar to the results in our previous publications in primary human bronchial epithelial cells (16, 17). Therefore, the NCI-H292 cell line was used as a valuable model to study the mechanisms (e.g., Sp1) underlying IL-13-induced MUC18 expression.

IL-13 increased MUC18 mRNA and protein in NCI-H292 cells transfected with a scrambled siRNA (control siRNA) (Figures 4A and 4B). However, Sp1 siRNA abrogated IL-13-induced MUC18 expression. Sp1 protein knockdown was confirmed using Western blot analysis (Figure 4B).

To demonstrate the functional consequences of Sp1 knockdown on bacterial adherence, NCI-H292 cells were infected with Mp for 24 hours. In IL-13-treated NCI-H292 cells, Sp1 siRNA significantly reduced Mp levels as compared with control siRNA (Figure 4C).

Involvement of MUC18 in Bacterial Adherence to Epithelial Cells

Having shown the up-regulation of MUC18 in asthmatic airway epithelium and determining the role of IL-13 in enhancing MUC18 expression, we determined the ability of MUC18 in binding bacteria. First, we used the MUC18-overexpressing HEK293 cell model to test the role of MUC18 in bacterial adherence because HEK293 cells, unlike primary human airway epithelial cells or NCI-H292 cells, express negligible levels of MUC18 (Figure 5A). MUC18 overexpression was confirmed in HEK293 cells transfected with a human MUC18 cDNA (Figure 5A). After 24 and 48 hours of incubation, cell-associated Mp levels in MUC18-overexpressing HEK293 cells were significantly higher than those in HEK293 cells without MUC18 overexpression (Figure 5B). Immunofluorescent staining on 2-μm-thick sections of embedded cells demonstrated that Mp was predominantly localized to the surface of MUC18-overexpressing HEK293 cells (Figure 5C). Second, to demonstrate that MUC18 may also bind to Mp in primary cells, well differentiated normal human bronchial epithelial cells infected with Mp under the ALI cultures were immunoprecipitated with an anti-MUC18 antibody, followed by Western blot using an anti-Mp antibody. Cells with Mp infection demonstrated a protein band of approximately 65 kD, suggesting an interaction of MUC18 and Mp in primary human airway epithelial cells (Figure 6A). Third, we assessed the direct binding of Mp to recombinant human MUC18 protein (*see details in the online supplement*). Figure 6B indicates the adherence of Mp to recombinant MUC18 protein.

Figure 4. Role of transcription factor specificity protein 1 (Sp1) in IL-13-induced MUC18 expression. NCI-H292 cells were transfected with a control siRNA or Sp1 siRNA for 48 hours, followed by cell culture medium (–) or IL-13 (10 ng/ml) treatment for 48 hours. (A) IL-13 treatment in control siRNA cells significantly increased MUC18 mRNA, which was abrogated by MUC18 siRNA. $n = 3$ replicates. (B) Western blotting of MUC18 and Sp1 demonstrated inhibition of IL-13-induced MUC18 up-regulation by Sp1 siRNA and reduction of Sp1 protein after Sp1 siRNA. $n = 3$ replicates. (C) Role of Sp1 in IL-13-induced *Mycoplasma pneumoniae* (Mp) adherence to NCI-H292 cells. Cells with a 48-hour transfection with a control siRNA or Sp1 siRNA were incubated with IL-13 (10 ng/ml) for 48 hours, followed by Mp infection (10 CFU/cell) for additional 24 hours. After three washes with PBS, cell-associated Mp was quantified. Sp1 siRNA, as compared with the control siRNA, significantly reduced cell-associated Mp levels. $n = 4$ replicates. Data are expressed as means $\pm$ SEM.

To test if MUC18 is involved in adherence to other species of bacteria such as NTHi, we first demonstrated that recombinant human MUC18 protein (versus BSA, an irrelevant protein control) coated on the 96-well plate increased NTHi binding (Figure 7A), suggesting the adherence of NTHi to recombinant MUC18 protein. Second, MUC18-overexpressing HEK293 cells were incubated with NTHi in the presence or absence of a human MUC18 neutralization antibody ABX-MA1. In the absence of ABX-MA1 (human IgG control only), cell-associated NTHi levels in MUC18-

Figure 5. MUC18 overexpression in HEK293 cells increased levels of cell-associated Mp. (A) MUC18 overexpression in HEK293 cells was confirmed using Western blotting. A total of 60 μ g of cell lysates from HEK293 cells with or without MUC18 cDNA transfection or 100 ng of recombinant human MUC18 (rhMUC18) protein (Abnova Corp., Walnut, CA) was run on a 8 to 16% gel and probed with a rabbit anti-MUC18 antibody (Epitomics, Inc., Burlingame, CA). (B) HEK293 cells with or without MUC18 overexpression were incubated with Mp (10 CFU/cell) for 24 or 48 hours. After three washes with PBS, cell-associated Mp levels were quantified. As compared with HEK293 cells without MUC18 overexpression, those with MUC18 overexpression demonstrated an increased level of cell-associated Mp. $n = 3$ replicates. Data are expressed as means $\pm$ SEM. (C) A fluorescent microscopic image showing increased Mp predominantly localized to the surface of MUC18-overexpressing cells. HEK293 cells with or without MUC18 overexpression were incubated with Mp (10 CFU/cell) for 24 hours, pelleted, embedded in glycol methacrylate resin, cut into sections at 2 μ m thickness, and stained with a rabbit anti-Mp antibody (Abcam, Cambridge, MA), followed by a FITC-labeled secondary antibody. Original magnification, $\times 400$.

overexpressing HEK293 cells were significantly higher than those in HEK293 cells without MUC18 overexpression (Figure 7B). In the presence of ABX-MA1, cell-associated NTHi levels in MUC18-overexpressing HEK293 cells, but not HEK293 cells without MUC18 overexpression, were significantly reduced (Figure 7B).

DISCUSSION

To the best of our knowledge, this is the first study focusing on the regulation and function of epithelial MUC18 in the context of a Th2 cytokine milieu and bacterial infection. We found that MUC18 expression in asthmatic airway epithelial cells was increased and that Th2 cytokine IL-13 consistently up-regulated MUC18 expression in primary human bronchial epithelial cells. Furthermore, MUC18 was shown to be involved in bacterial (e.g., Mp and NTHi) adherence to epithelial cells.

Most subjects with asthma in the current study were treated with inhaled corticosteroids (ICS), whereas normal control subjects were not. To determine whether ICS alter MUC18 expression in airway epithelial cells, primary normal human bronchial epithelial cells ($n = 2$) under ALI cultures were treated with fluticasone propionate (10^{-7} M) prepared in 0.1% DMSO or 0.1% DMSO (control) for 48 hours. Fluticasone propionate did not significantly change the MUC18 mRNA expression. Thus, ICS may not affect airway epithelial MUC18 expression in patients with asthma.

Previous studies have centered on investigating the expression and function of MUC18 in malignant melanoma and

prostate cancer (4, 6, 27). As a result, MUC18 up-regulation has been considered as a biomarker of cancer development, such as metastasis. However, little is known about MUC18 regulation in nonmalignant conditions. An early study suggested MUC18 up-regulation in bronchial epithelial cells from patients with COPD (7). Although that was the first study to evaluate MUC18 expression in a lung disease, the function of MUC18 in airway mucosa and the underlying mechanisms of MUC18 up-regulation have not been explored.

Our current study focused on the impact of a Th2 cytokine milieu on MUC18 expression because increased Th2 cytokines are not only seen in allergic diseases such as asthma but are also implicated in several inflammatory diseases, including COPD and ulcerative colitis (11, 28). Our initial finding of increased MUC18 expression in fatal asthmatic airway tissue (e.g., epithelium) was later confirmed in brushed bronchial epithelial cells that were directly obtained from subjects with mild to moderate asthma. Moreover, in cultured, well differentiated bronchial epithelial cells of normal subjects and subjects with asthma, IL-13 directly increased MUC18 mRNA levels, particularly in cells from normal subjects. Like our previous studies examining other parameters (e.g., mucin MUC5AC) in airway epithelial cells (19, 29), normal subjects or subjects with asthma demonstrated individual variations in MUC18 expression levels.

Unlike MUC18 mRNA, epithelial MUC18 protein was only significantly increased in asthmatic cells. The mechanisms for such a discrepancy between MUC18 mRNA and protein expression in normal airway epithelial cells remain unknown and warrant future investigation, including examination of posttranscriptional

Figure 6. MUC18 interactions with Mp. (A) Cell lysates of primary normal human bronchial epithelial cells infected with Mp (10 CFU/cell) under air-liquid interface conditions for 7 days were immunoprecipitated with a rabbit anti-MUC18 antibody (Epitomics, Inc., Burlingame, CA) or a rabbit IgG (IgG control) (Abcam, Cambridge, MA). Coprecipitating Mp proteins were detected using an anti-Mp antibody (Abcam). A protein around 65 kD in Mp was shown to interact with MUC18. (B) Direct binding assay of recombinant human MUC18 protein and Mp. A full-length recombinant human MUC18 protein (1 μ g/ml) (Abnova Corp., Walnut, CA) or BSA (1 μ g/ml, control for MUC18 protein) was coated on a 96-well plate. After washing the plate, Mp (10⁶ CFU in 50 μ l PBS) was added. After three washes, a rabbit anti-Mp antibody (Abcam) was added, followed by incubation with a biotinylated anti-rabbit secondary antibody and avidin-biotin-peroxidase. Tetramethylbenzidine was used as a substrate to develop the plate, and the absorbance at 450 nm was obtained. The increased absorbance at 450 nm indicated a direct binding of MUC18 protein to Mp. $n = 3$ replicates. Data are expressed as means $\pm$ SEM.

modification of MUC18 mRNA in normal versus asthmatic epithelial cells. Nonetheless, consistent up-regulation of MUC18 mRNA and particularly protein in asthmatic cells suggests that MUC18 may be an important regulator of impaired airway mucosal function in asthma. To increase the relevance of IL-13-induced MUC18 in cultured human bronchial epithelial cells to asthmatic airways, we propose to collect endobronchial biopsy samples in future studies to simultaneously examine IL-13 expression and MUC18 in bronchial mucosa.

How IL-13 regulates MUC18 is unclear. Previous studies in malignant melanoma cells indicate that several transcription factors, including Sp1, are able to bind to MUC18 promoter. Using the RNA interference approach in NCI-H292 cells, we demonstrated that Sp1 knockdown significantly reduced IL-13-induced MUC18 expression, which is accompanied by less bacterial (e.g., Mp) adherence to the epithelial cells. Because Sp1 knockdown did not completely block MUC18 induction after IL-13 treatment, it is likely that other transcription factors, such as activator protein-2, may also be involved. This possibility will be explored in our future experiments. One limitation in the current study is that we studied the role of Sp1 only in NCI-H292 cells but not in the well differentiated primary human airway epithelial cells because transfection experiments for siRNA are much easier in NCI-H292 cells than in primary cells.

One of the key unanswered questions is about the function of MUC18 in nonmalignant settings. In physiological conditions, MUC18 is considered as an intercellular adhesion molecule of cells like endothelial cells (3). Studies in human malignant melanoma

Figure 7. MUC18 interactions with nontypeable *Haemophilus influenzae* (NTHi). (A) Direct binding assay of recombinant human MUC18 protein and NTHi was similarly performed as described for Figure 6B. The increased absorbance at 450 nm indicated a direct binding of MUC18 protein to NTHi. $n = 4$ replicates. Data are expressed as means $\pm$ SEM. (B) NTHi association with MUC18-overexpressing HEK293 cells. HEK293 cells with or without MUC18 overexpression were incubated with a control IgG or an anti-human MUC18 antibody ABX-MA1 (10 μ g/ml) for 2 hours, followed by infection with NTHi (10 CFU/cell) for 24 hours. After three washes with PBS, cell-associated NTHi was quantified. As compared with HEK293 cells without MUC18 overexpression, MUC18-overexpressing HEK293 cells demonstrated an increased level of cell-associated NTHi, which was blocked by the anti-MUC18 antibody treatment. $n = 3$ replicates. Data are expressed as means $\pm$ SEM.

strongly suggest that MUC18 is critical to tumor progression, such as metastasis (30). Our study is the first to suggest that one of the novel functions for MUC18 is to participate in bacterial adherence to epithelial cells. First, by using human MUC18-overexpressing HEK293 cells, we demonstrated that human MUC18 protein is involved in cell adherence of two of the most common bacteria (Mp and NTHi) in chronic lung diseases. Second, in well differentiated primary human bronchial epithelial cells, we found that MUC18 may directly interact with Mp, possibly through a protein at 65 kD. The identity of this 65-kD protein needs to be revealed in our future studies. Based on a previous publication (31), we speculate that this 65-kD protein could be the P65, a cytoadherence-associated protein in Mp. The role of MUC18 in bacterial adherence to primary human bronchial epithelial cells needs to be carefully examined because multiple epithelial molecules could contribute to bacterial adhesion. Whether the effects of MUC18 on Mp or NTHi adherence are indirect or direct remain to be further explored. Nonetheless, the data shown in Figures 6B and 7A suggest the adherence of Mp or

NTHi to recombinant human MUC18 protein. The bacterial components (e.g., adhesins) used by both strains of bacteria involved in MUC18 binding need to be identified in future experiments. The components in different bacterial strains may not necessarily be identical because previous studies suggest that different adhesins from various bacteria (e.g., NTHi and *M. catarrhalis*) could bind the same host cell molecule/receptor, such as carcinoembryonic antigen-related cell adhesion molecule 1 (32).

Our current study has revealed a novel function (e.g., bacterial adherence) of MUC18, but there are still several pivotal questions to be addressed. For example, does MUC18 contribute to the infectious process of other strains of respiratory bacteria (e.g., *Pseudomonas aeruginosa*)? If MUC18 is involved in a broad-spectrum of bacterial infection, there will be an urgency to develop MUC18-targeted therapy to attenuate pathogenic bacterial infections in lung diseases such as asthma, COPD, and cystic fibrosis. Another relevant question is regarding the role MUC18 in inflammatory processes after bacterial adherence to the epithelial cells. Because MUC18 engagement in endothelial cells initiates an outside-in signaling pathway involving the protein tyrosine kinases FYN and FAK as well as Paxillin (33), it is conceivable that MUC18-bacterial interaction may lead to activation of intracellular signaling pathways, which may in turn modulate inflammatory responses to bacteria. Therefore, we foresee a new era of functional studies of MUC18 in airway epithelial innate immunity.

In summary, our work challenges the existing concept that MUC18 is most likely up-regulated in malignant diseases. Results from the current study strongly suggest that MUC18 can be increased in airway epithelial cells under a nonmalignant condition such as a Th2 cytokine milieu. Furthermore, MUC18 was shown to be involved in respiratory bacterial adherence to epithelial cells. Together, our findings may provide a broad-spectrum therapeutic approach to treat respiratory infections of various strains of bacteria in pulmonary diseases.

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