[Frontiers in Bioscience E2, 68-77, January 1, 2010]

MUC1 expression by human airway epithelial cells mediates *pseudomonas aeruginosa* adhesion

Kosuke Kato[1,2], Erik P. Lillehoj[3], Hirofumi Kai[2], Kwang Chul Kim[1]

[1]Department of Physiology and Lung Center, Temple University School of Medicine, Philadelphia, PA, [2]Department of Molecular Medicine, Kumamoto University, Kumamoto, Japan, [3]Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD

TABLE OF CONTENTS

1. ABSTRACT

Human MUC1 (Muc1 in animals) is an extensively O-glycosylated membrane-tethered mucin expressed on the surface of epithelial cells and some cells of the hematopoietic system. Recently, we showed that the hamster Muc1 on Chinese hamster ovary (CHO) cells served as a binding site for *Pseudomonas aeruginosa* (PA) through interaction between bacterial flagellin and the Muc1 ectodomain. Because CHO cells are known to produce an atypical pattern of protein glycosylation, we determined whether or not PA interacted with MUC1 endogenously expressed on human airway epithelial cells. Knock down of MUC1 expression in bronchial (NuLi-1) or alveolar (A549) epithelial cells by RNA interference significantly reduced PA binding to the cells. Conversely, over-expression of MUC1 in HEK293 cells increased bacterial adherence. By confocal microscopy, PA and MUC1 were colocalized on the surface of NuLi-1 cells. Taken together, these results confirm our previous observations in CHO cells and suggest that MUC1 serves as a binding site for PA on the surface of airway epithelial cells, which may have important consequences in the pathogenesis of PA lung infections.

2. INTRODUCTION

Pseudomonas aeruginosa (PA) is a Gram-negative bacterium that is commonly found in soil and water, but also is an opportunistic pathogen in patients who are predisposed to infection due to mechanical ventilation or immunosuppression, or who possess a homozygous mutant CFTR genotype. As a cause of ventilator-associated pneumonia, PA has a higher mortality compared with other bacterial pathogens (1). In the community, the incidence of PA respiratory tract infections is increasing in nursing home residents and patients with chronic obstructive pulmonary disease (2). In addition, PA is the most prevalent chronic lung infection in patients with cystic fibrosis (CF) (3). Therefore, elucidating the mechanisms through which PA establishes and maintains lung colonization in the milieu of host inflammation will greatly contribute to understanding the etiopathogenesis of respiratory diseases.

A central dogma in the colonization of epithelial tissues by pathogens is that the interaction between microbial adhesins and their cognate host cell receptors is essential for establishment of infection (4). Because PA is

an important respiratory pathogen, major efforts have been directed at identifying the microbial and host factors relevant to initial bacterial attachment to airway cells. In this context, we noted with interest the prior reports that mucin glycoproteins purified from the sputum of CF patients were tightly bound to PA bacteria, suggesting that mucins act as bacterial receptors in the airways (5, 6). However, these studies did not address the relationship between PA binding to the gel forming mucins and the host response to the bacteria at the molecular and cellular levels.

Our laboratory had previously characterized a primary hamster tracheal surface epithelial (TSE) cell culture system as a model to study the biochemistry and pharmacology of mucin secretion (7). Mucin secretion was stimulated by neutrophil elastase, and biochemical and histochemical studies revealed that a significant portion of the released mucins was derived from the cell surface (8). Because the only cell-associated mucin known at that time was MUC1, which had been cloned from a breast cancer cell line (9), we speculated that hamster TSE cells expressed Muc1. (By convention, MUC designates the human mucins and Muc refers to non-human homologues.) This was later confirmed by molecular cloning of the hamster Muc1 gene (10) and identification of Muc1 in the plasma membrane fraction and on the apical surface of the TSE cells (11).

It is now well established that MUC1 is expressed on the apical surface of most airway epithelial cells, including goblet cells, ciliated cells, and types I and II alveolar pneumocytes (12, 13). The deduced amino acid sequence of the MUC1 gene indicates a three domain structure of the protein with an extended, highly glycosylated NH_2-terminal extracellular (EC) domain, a single pass transmembrane (TM) domain, and a COOH-terminal cytoplasmic tail (CT) domain (9). The MUC1 EC region contains a 20-amino acid tandem repeated segment with O-linked glycan moieties. The MUC1 CT domain is encoded by 72 amino acids including seven tyrosine residues, some of which are phosphorylated leading to activation of intracellular signal transduction cascades (14). On the basis of these structural attributes, and the ability of CF mucins to bind to PA, we proposed that MUC1 serves as a receptor for PA in the airways (15). In support of this hypothesis, it was observed that PA exhibited significantly greater adhesion to Chinese hamster ovary (CHO) cells stably expressing the cloned hamster Muc1 gene compared with cells transfected with the corresponding empty vector, suggesting a direct bacterial interaction with the glycosylated Muc1 ectodomain (16). However, given that MUC1 over-expressed in CHO cells exhibits an uncommon pattern of O-glycosylation (17), and the fact that the tandem repeats of human MUC1 (GSTAPPAHGVTSAPDTRPAP) and hamster Muc1 (GSSAPVTSSATNAPTTPVHS) only share 8 common residues (underlined), it was necessary to verify these observations using MUC1 expressed by human airway epithelial cells. In the current report, we show that MUC1 endogenously expressed by human bronchial and alveolar epithelial cells serves as a binding site for PA. These results are discussed in relation to MUC1's ability to counter-regulate the innate pro-inflammatory response, thereby preventing excessive inflammation during the latter stages of PA airway infection.

2. MATERIALS AND METHODS

2.1. Reagents

All reagents were from Sigma (St. Louis, MO) unless noted otherwise. Anti-MUC1 antibody (GP1.4) was from Biomeda (Foster City, CA). A rabbit antiserum (CT33) against a synthetic peptide corresponding to the COOH-terminal 17 amino acids of MUC1 has been described (18, 19). Horseradish peroxidase (HRP)-conjugated goat anti-mouse and goat anti-rabbit IgG antibodies were from KPL (Gaithersburg, MD). Alexa Fluor 488-conjugated rabbit anti-mouse IgG and Alexa Fluor 555-goat anti-mouse IgG were from Invitrogen (Carlsbad, CA). The MUC1-pcDNA3.1 expression plasmid was a generous gift from Dr. Sandra J. Gendler (Mayo Clinic, Scottsdale, AZ).

2.2. Cells

A549, an alveolar type II cell line derived from a lung adenocarcinoma (20), was obtained from the American Type Culture Collection (Manassas, VA) and cultured as recommended. NuLi-1 cells, generously provided by Dr. Joseph Zabner (University of Iowa, Iowa City, IA), were derived from normal airway epithelium and immortalized by infection with HPV-16 E6/E7 (21). The cells were cultured on collagen-coated plastic dishes (type VI, human placental; Sigma) in serum-free bronchial epithelial cell growth medium with supplements (Lonza/Cambrex Bioscience, Walkersville, MD). HEK293 cells stably expressing MUC1 in the pcDNA3.1 vector (MUC1-HEK293) or empty vector alone (pcDNA3.1-HEK293) have been described (19) and were cultured in DME medium, 10% FBS, antibiotics, and 800 μg/ml of G418 (Invitrogen).

2.3. MUC1 siRNA transfection

NuLi-1 (2.0×10^6) and A549 (1.0×10^6) cells were seeded in 12- or 24-well plates, incubated for 24 hr, and transfected with 5 or 20 nM of a MUC1 siRNA or a nontargeting control siRNA (D-001210, Dharmacon, Lafayette, CO) using 1.0 or 2.0 μl/well of Lipofectamine2000 (Invitrogen) according to the manufacturer's instructions. The MUC1 siRNA consisted of a 21-bp sequence derived from the MUC1 gene (22). Knock down of MUC1 mRNA and protein was confirmed by real time RT-PCR and Western blotting (23).

2.4. *Pseudomonas aeruginosa*

PA strain K (PAK) is a nonmucoid, piliated, and motile strain. PAO1 is a mucoid, piliated and motile strain. PAO1 expressing green fluorescent protein (GFP) (24) was kindly provided by Dr. Joanna B. Goldberg (University of Virginia, Charlottesville, VA). Bacteria were cultured overnight in LB broth supplemented with carbenicillin (60 μg/ml), washed with PBS, and cell concentrations determined spectrophotometrically using an OD_{600} of 0.5 = 5.0×10^8 cells.

2.5. PA binding assays

Adhesion of PA to cells was performed by two assay procedures. The first utilized ^{35}S-labeled bacteria according to the method described (16). Briefly, the cells were treated with the MUC1 siRNA or control siRNA as described above, washed twice with PBS, fixed for 10 min with 2.5% glutaraldehyde in PBS at room temperature, and washed three times with PBS. PAK were metabolically radiolabeled in sulfate-free M9 medium containing 10 μCi/ml $Na_2{}^{35}SO_4$ (1,175 Ci/mmol, 100 mCi/ml, carrier-free; American Radiolabeled Chemicals, St. Louis, MO) for 16 hr at 37°C, washed twice, resuspended in PBS containing 2.0 mg/ml glucose, and quantified. Fixed cells were with various colony forming units (CFU) of PAK in 0.5 ml for 40 min at 37°C, washed three times with PBS, adhered bacteria were lysed with 1.0 ml of 2% sodium dodecyl sulfate, and radioactivity was measured by liquid scintillation counting. In the second procedure, GFP-PAO1 bacteria were incubated with fixed cells, unbound bacteria were removed by washing, and GFP fluorescence of adhered bacteria was detected by fluorescence-activated cell sorting (FACS).

2.6. FACS

The cells detached with 0.02% EDTA in PBS, were washed with PBS containing 1% FBS (wash buffer), resuspended in ice-cold wash buffer and either directly analyzed by FACS (for GFP-PAO1 binding assay) or incubated in wash buffer containing anti-MUC1 antibody (GP1.4; 1:100 dilution), and incubated for 30 min on ice. After washing, the cells were resuspended in ice-cold wash buffer containing Alexa Fluor 488-conjugated rabbit anti-mouse IgG secondary antibody (Invitrogen, 1:100) and incubated for 30 min on ice. The cells were washed, fixed with 1% paraformaldehyde at 4°C, and analyzed with a FACSCalibur instrument using BD FACS Comp software (BD Biosciences, Palo Alto, CA).

2.7. Immunofluorescence

NuLi-1 cells were grown on 8-well glass slides (Nalge Nunc, Naperville, IL), exposed to GFP-PAO1 for 10 min, the cells were washed 5 times with PBS, and fixed with 4% paraformaldehyde. The fixed cells were incubated with 5% goat serum (Abcam, Cambridge, MA) for 20 min, and sequentially incubated with GP1.4 antibody (1:100 in 1% goat serum) for 1 hr and Alexa Fluor 555-goat anti-mouse IgG (1:500 in 1% goat serum) for 30 min at room temperature. The cells were mounted with anti-fade reagent with DAPI (Invitrogen) and visualized with a Zeiss LSM 510 Meta confocal microscopy (Zeiss, Oberkochen, Germany) under 63X oil objective.

2.8. Statistical analysis

Differences between mean ± SEM values of various treatment groups were compared using the Student's t-test and considered significant at $p < 0.05$.

4. RESULTS

4.1. Knock down of MUC1 expression inhibits PA binding to human airway epithelial cells

Our prior results demonstrated that both PAK and PAO1 adhered to CHO cells stably transfected with a hamster Muc1 cDNA (CHO-Muc1) to a significantly greater extent compared with Muc1 non-expressing CHO cells (16). To extend these studies to human airway epithelial cells endogenously expressing MUC1, we took the approach of blocking MUC1 expression by RNA interference and comparing PA binding to these cells with the corresponding MUC1 expressing cells. The MUC1 siRNA significantly reduced MUC1 mRNA levels (Figure 1A) and decreased protein expression both in cell lysates (Figure 1B) and on the surface (Figure 1C) of NuLi-1 cells. By FACS analysis, 5.3% of NuLi-1 cells transfected with the MUC1 siRNA stained positive with an antibody (GP1.4) against the MUC1 ectodomain, compared with 83% of the cells treated with a non-targeting control siRNA. Identical results were seen using siRNA-treated A549 cells (Figure 1D).

Two PA binding assays were used to compare bacterial adhesion to the cells transfected with the MUC1 siRNA or control siRNA. The first utilized ^{35}S-labeled PAK and glutaraldehyde-fixed NuLi-1 cells according to the previous procedure using CHO-Muc1 cells (16). As shown in Figure 2, PA binding to NuLi-1 cells increased with increasing input CFU of PAK and cells treated with the MUC1 siRNA exhibited significantly decreased adhesion compared with cells transfected with the control siRNA at the two highest bacterial doses. In the second approach, NuLi-1 cells were transfected with the MUC1 siRNA or control siRNA, the fixed monolayers were untreated or incubated with GFP-PAO1, following which the cells were extensively washed, detached, and analyzed by FACS for GFP staining. The bronchial epithelial cells treated with the control siRNA displayed a GFP-PAO1 dose-dependent increase in immunofluorescence staining (Figure 3). By contrast, treatment of the cells with the MUC1 siRNA reduced bacterial adhesion to the level of the PA untreated negative control. Similarly, A549 cells transfected with the MUC1 siRNA demonstrated reduced PA adhesion compared with cells transfected with the control siRNA (Figure 4).

4.2. Over-expression of MUC1 in HEK293 cells augments PA binding

To confirm the interaction between PA and MUC1 in an independent cell culture system, the GFP-PAO1 adherence assay was repeated using MUC1 non-expressing HEK293 cells that had been stably transfected with a MUC1 cDNA, or empty pcDNA3.1 vector as a negative control. Confocal microscopy and Western blotting verified MUC1 expression in MUC1-HEK293, but not pcDNA3.1-HEK293 cells (Figures 5A and 5B). Incubation of GFP-PAO1 with the cells for 1 hr followed by FACS analysis revealed approximately 3-fold increased bacterial adherence to MUC1-HEK293 cells compared with pcDNA3.1-HEK293 cells (Figure 5C).

4.3. Colocalization of PA and MUC1 on the cell surface

Laser scanning confocal microscopy was performed to confirm the localization of PA on the cell surface in the context of MUC1 expression. In non-permeabilized NuLi-1 cells, MUC1 exhibited a staining pattern primarily localized to the cell surface (Figure 6A).

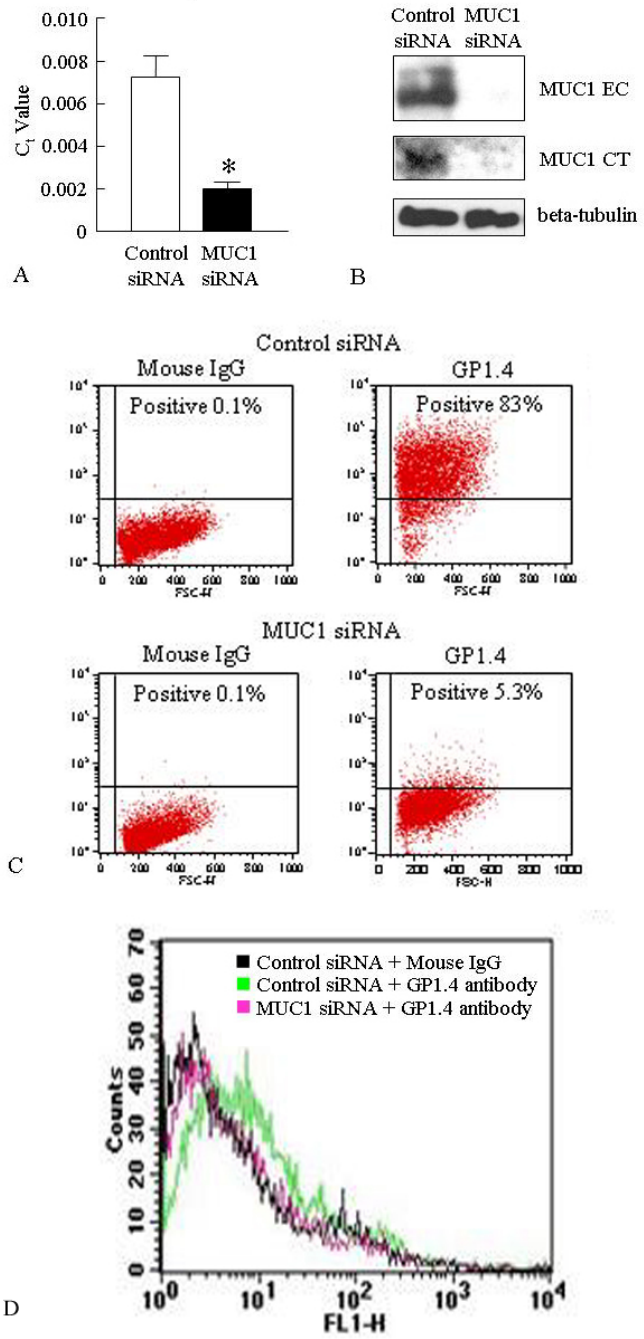

Figure 1. MUC1 siRNA decreases MUC1 expression in NuLi-1 human airway epithelial cells. NuLi-1 cells were seeded at 2.0 x 10^6 cells/well in 12-well plates, cultured for 24 hr, and transfected with 5 nM of a control siRNA or MUC1 siRNA using 2.0 µl/well of Lipofectamine2000. (A) MUC1 mRNA levels were quantified by real time RT-PCR at 72 hr post-transfection. Each bar represents the mean ± SEM values (n = 3). The Ct value was defined as the number of PCR cycles required for the specific fluorescence signal to exceed the detection threshold value set by the software installed in the iCycler. The levels of *MUC1* transcripts were normalized to *GAPDH* transcripts using the $-2^{\Delta\Delta Ct}$ method. *, significantly decreased mRNA level ($p < 0.05$). (B) MUC1 protein levels in cell lysates were detected by immunoblotting with MUC1 EC antibody GP1.4 and MUC1 CT antibody CT33. Immunoblotting for beta-tubulin confirmed equal sample loadings. (C) MUC1 protein expression on the cell surface was detected by FACS analysis with GP1.4 and Alexa Fluor 488-conjugated rabbit anti-mouse IgG secondary antibody. (D) A549 cells were seeded at 1.0 x 10^6 cells/well in 12-well plates and transfected as above except that 20 nM of siRNAs were used. At 72 hr post transfection, cell surface expression levels of MUC1 were determined with the GP1.4 MUC1 EC antibody by FACS.

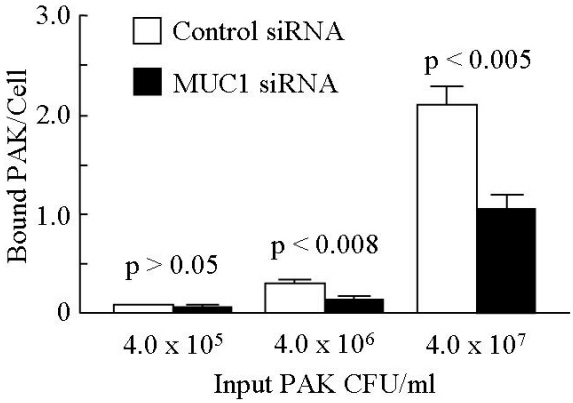

Figure 2. Transfection of NuLi-1 cells with MUC1 siRNA inhibits PAK binding. NuLi-1 cells were seeded at 2.0 x 10^6 cells/well in 24-well plates, cultured for 24 hr, and transfected with 5 nM of a control siRNA or MUC1 siRNA using 1.0 µl/well of Lipofectamine2000. At 72 hr post-transfection, the cells were fixed with 2.5% glutaraldehyde, incubated for 40 min at 37°C with the indicated input CFU of ^{35}S-labeled PAK in a total volume of 0.5 ml, the cells were washed to remove unbound bacteria, and PAK remaining in the wells determined by liquid scintillation counting. Each bar represents the means ± SEM (n = 3). The p values comparing control siRNA vs. MUC1 siRNA transfections for each input CFU of PA are indicated.

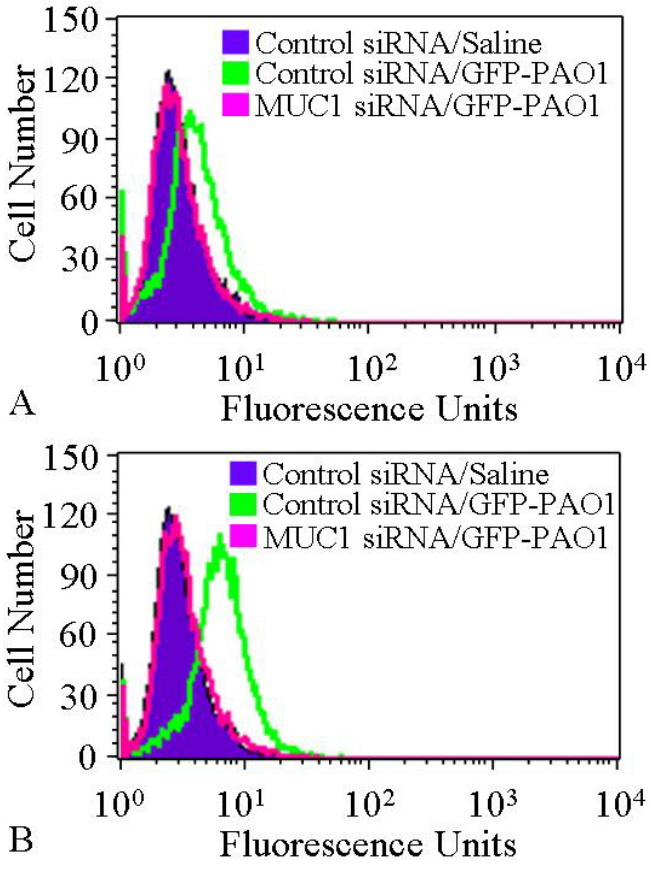

Figure 3. Transfection of NuLi-1 cells with MUC1 siRNA inhibits GFP-PAO1 binding. NuLi-1 cells were seeded at 2.0 x 10^6 cells/well in 12-well plates, cultured for 24 hr, and transfected with 5 nM of a control siRNA or MUC1 siRNA using 2.0 µl/well of Lipofectamine2000. At 72 hr post-transfection, the fixed-cells were untreated or incubated for 40 min at 37°C with 4.0 x 10^7 (A) or 4.0 x 10^8 (B) CFU of GFP-PAO1 in total volume of 1.0 ml, the cells were washed to remove unbound bacteria, and GFP-PAO1 remaining attached to the cells was determined by FACS analysis. Data were obtained from a total of 1 x 10^4 viable cells for each analysis. Results are representative of 3 experiments.

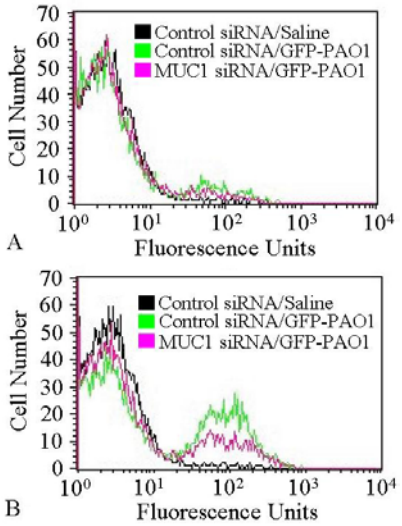

Figure 4. Transfection of A549 cells with MUC1 siRNA inhibits GFP-PAO1 binding. A549 cells were seeded at 1.0 x 10^6 cells/well in 12-well plates, cultured for 24 hr, and transfected with 20 nM of a control siRNA or MUC1 siRNA using 2.0 µl/well of Lipofectamine2000. At 72 hr post-transfection, the fixed-cells were untreated or incubated for 60 min at 37°C with 4.0 x 10^6 (A) or 4.0 x 10^7 (B) CFU of GFP-PAO1 in total volume of 1.0 ml, the cells were washed to remove unbound bacteria, and GFP-PAO1 remaining attached to the cells was determined by FACS analysis. Data were obtained from a total of 1 x 10^4 viable cells for each analysis. Results are representative of 3 experiments.

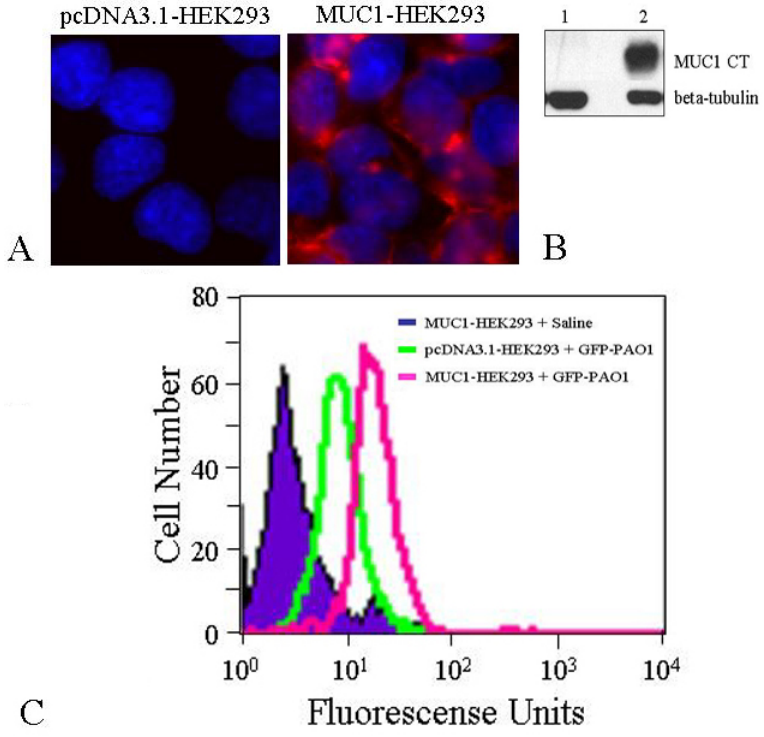

Figure 5. Over-expression of MUC1 in HEK293 cells increases GFP-PAO1 binding. (A) Confocal microscopy of pcDNA3.1-HEK293 and MUC1-HEK293 cells. (B) Equal protein aliquots of lysates of pcDNA3.1-HEK293 (lane 1) and MUC1-HEK293 (lane 2) cells were analyzed by immunoblotting with MUC1 CT antibody CT33. Immunoblotting for beta-tubulin confirmed equal sample loadings. (C) pcDNA3.1-HEK293 and MUC1-HEK293 cells were fixed with 2.5% glutaraldehyde, incubated for 1 hr at 37°C with saline or 4.0 x 10^7 CFU of GFP-PAO1, the cells were washed to remove unbound bacteria, and GFP-PAO1 remaining attached to the cells was determined by FACS analysis. Data were obtained from a total of 1 x 10^4 viable cells for each analysis. Results are representative of 3 experiments.

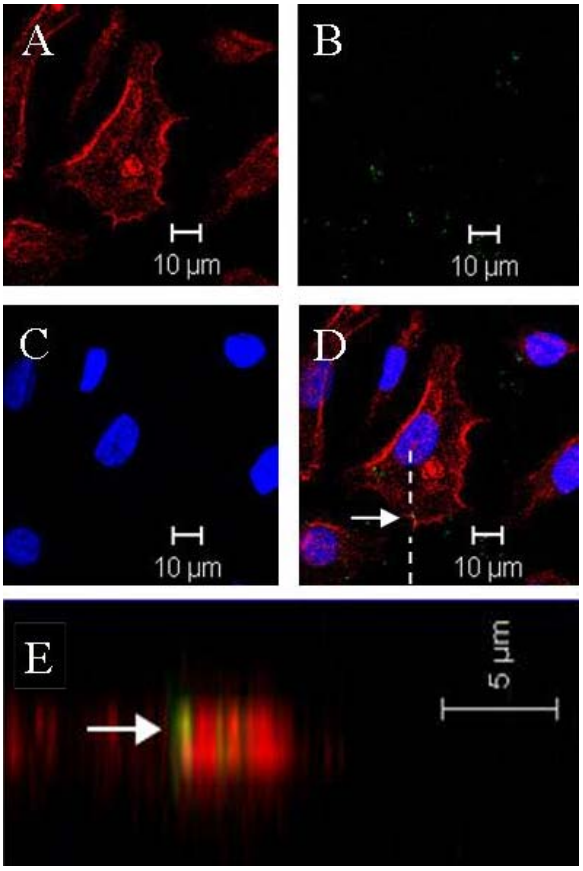

Figure 6. Colocalization of GFP-PAO1 and cell surface MUC1 on NuLi-1 cells. NuLi-1 cells were cultured in 8-well chambers, incubated with 1.0 x 10^7 CFU of GFP-PAO1 for 30 min at 37°C, washed 5 times with PBS, fixed with 4% paraformaldehyde, and stained with GP1.4 antibody plus Alexa Fluor 555-conjugated secondary antibody. The cells were visualized by confocal microscopy as follows: (A) GP1.4 antibody (red); (B) GFP-PAO1 (green); (C) DAPI staining of nuclei (blue); (D) overlay of (A), (B) and (C); (E) overlay of (A), (B), and (C) and visualized by z stack imaging. The colocalization of GFP-PAO1 with MUC1 is indicated by yellow staining of the merged images (arrows in (D) and (E)). The dashed line in (D) shows the section used for z stack imaging in (E).

Incubation of the cells with GFP-PAO1 for 30 minutes followed by extensive washing to remove unbound bacteria revealed the presence of PA bound to the cell surface (Figure 6B). Colocalization of GFP-PAO1 with MUC1 was observed after incubation of NuLi-1 cells with bacteria, upon both x-y imaging (Figure 6D) and z-stack imaging (Figure 6E). As a negative control, no GFP staining was observed with non-treated NuLi-1 cells (data not shown).

DISCUSSION

In this study, we demonstrated that knock down of MUC1 expression in NuLi-1 bronchial epithelial or A549 alveolar epithelial cells by RNA interference significantly reduced PA adherence to the cells. Over-expression of MUC1 in HEK293 cells increased PA binding to the cell surface. Finally, by confocal microscopy, PA and MUC1 were colocalized to the surface of NuLi-1 cells. Taken together, these results suggest that MUC1 serves as a binding site for PA on the surface of human airway epithelial cells.

We considered the possibility that another PA receptor whose expression is modulated by MUC1 was responsible for bacterial adhesion. However, two lines of evidence are inconsistent with this hypothesis. First, PA binding to CHO cells expressing a Muc1 deletion mutant lacking the entire EC domain, but retaining the TM and CT regions of the molecule, was reduced to the level of that seen using Muc1 negative cells, indicating that the EC domain alone was necessary and sufficient for bacterial adhesion (16). Second, treatment of A549 cells with PA bacteria or its purified flagellin induced tyrosine phosphorylation of the MUC1 CT (unpublished observations). The latter effect is reminiscent of the published studies demonstrating that treatment of cells expressing a CD8/MUC1 chimeric protein (containing the CD8 ectodomain and TM region fused to the MUC1 CT) with anti-CD8 antibody stimulated tyrosine phosphorylation of the CT (25, 26).

PA is an opportunistic pathogen responsible for a wide range of pulmonary infections, one of the most debilitating being chronic infection and inflammation in CF patients. Transiently inspired PA are normally trapped by airway mucus and removed by mucociliary clearance, but pulmonary clearance is impaired in CF lungs. Although the reasons for impaired PA clearance in CF remain to be determined, several mechanisms have been proposed. These include increased bacterial adhesion to CF airway epithelial cells compared with non-CF cells (27, 28), conversion of non-mucoid strains of PA that initially colonize the upper respiratory tract into mucoid alginate-producing variants (29), mucus hypersecretion and plugging of the small airways (30), over-activation of intracellular signaling pathways (31), and an exaggerated host inflammatory response (32, 33). DiMango and coworkers (34) demonstrated that asialo-GM1 glycolipids on the surface of airway epithelial cells were responsible for adhesion of PA. These authors also showed that airway epithelial cells from CF patients showed higher levels of asialo-GM1 suggesting a mechanism whereby PA colonization of CF lungs leads to chronic infection and inflammation as a result of increased bacterial adhesion early in the course of disease. It is important to note, however, that the molecular interactions between PA and respiratory epithelial cells are complex, involving multiple types of ligand-receptor contacts, and the relevance of any particular ligand-receptor interaction in bacterial adhesion is controversial (35). For example, although several glycolipids, including asialo-GM1, have been suggested to act as co-receptors with TLR2 and TLR5 for flagellin (36, 37), a subsequent study disputed this claim (38).

A variety of PA-associated macromolecules are TLR agonists that could potentially serve to stimulate

airway inflammation in CF (36). However, only a limited number of studies have examined the TLR-mediated inflammatory response in the airways and, in particular, the role of PA-dependent inflammation in CF. In normal airways, TLR5 is expressed both by alveolar macrophages and epithelial cells (36, 39) and one study showed that a dominant TLR5 stop codon polymorphism abolished flagellin signaling that was associated with increased susceptibility to Legionnaires' disease (40). Treatment of airway epithelial cells with PA flagellin induced TLR5 gene expression and mobilized the receptor from the basolateral to the apical surface of the cells (36). Although CF lung cells have been shown to express equal amounts of TLR5 mRNA and protein compared with non-CF cells (41), the effects of PA or flagellin on TLR5 expression and function in CF airways have not been reported.

TLR signaling is activated by ligand-induced receptor aggregation, recruitment of cytoplasmic adaptor proteins (MyD88, TIRAP, TRIF) to the Toll/interleukin-1 receptor (TIR) intracellular domain, and activation of downstream kinases (IRAK, TRAF6) (42). In the case of TLR5, binding of bacterial flagellin activated NF-kappaB, PI3K/Akt, and mitogen-activated protein kinases (36, 43-45). Most of these downstream components also contribute to the innate immune response following ligation of other members of the TLR family with their cognate agonists. Indeed, our recent study using both *in vivo* and *in vitro* systems demonstrated that intracellular signaling cascades activated by TLR2, 3, 4, 5, 7, and 9 were suppressed by MUC1 (46).

In conclusion, based on the results presented in this report, as well those of prior publications, we suggest the following model of innate immune mechanisms in the airways following exposure to potential infectious agents such as PA. Under normal conditions, the small number of PA entering the lungs are trapped in airway surface liquid and quickly removed by mucociliary clearance and phagocytosis. If the numbers of PA increase, for example due to a predisposing condition such cystic fibrosis, TLRs on epithelial cells and resident macrophages are activated resulting in production of inflammatory mediators that promote leukocyte influx into the airways. We propose that the low level of MUC1 expression is insufficient to antagonize TLR signaling, but during ongoing inflammation the level of MUC1 expression increases by the direct effect of pro-inflammatory cytokines (47, 48), as well as neutrophil elastase which is released by infiltrating neutrophils and up-regulates MUC1expression (23). As a result of increased levels of MUC1, TLR-induced inflammation is inhibited, thus protecting the lung from excessive inflammation. Intuitively, one might expect that greater MUC1 protein expression may also increase PA adherence to airway epithelial cells through binding to the MUC1 ectodomain. We (49) and others (14), however, have previously speculated that the presence of the extracellular cleavage site allows the MUC1 ectodomain to be shed from the cell surface, thereby releasing any bound constituents from the epithelium. Current studies in our laboratory are underway to elucidate the relative roles of the anti-inflammatory and bacterial binding-release functions of MUC1 in the airways.

6. ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service grants AI-72291 (EPL), HL-47125 and HL-81825 (KCK), and the Cystic Fibrosis Foundation (EPL).

7. REFERENCES

1. J. Chastre and J.Y. Fagon. Ventilator-associated pneumonia. *Am J Respir Crit Care Med* 165, 867-903 (2002)

2. M. Ruiz, S. Ewig, A. Torres, F. Arancibia, F. Marco, J. Mensa, M. Sanchez, J.A. and Martinez. Severe community-acquired pneumonia. Risk factors and follow-up epidemiology. *Am J Respir Crit Care Med* 160, 923-929 (1999)

3. J.B. Lyczak, C.L. Cannon and G.B. Pier. Lung infections associated with cystic fibrosis. *Clin Microbiol Rev* 15, 194-222 (2002)

4. E.H. Beachey. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surface. *J Infect Dis* 143, 325-345 (1981)

5. R. Ramphal, C. Guay and G.B. Pier. *Pseudomonas aeruginosa* adhesins for tracheobronchial mucin. *Infect Immun* 55, 600-603 (1987)

6. C. Carnoy, A. Scharfman, E. Van Brussel, G. Lamblin, R. Ramphal and P. Roussel. *Pseudomonas aeruginosa* outer membrane adhesins for human respiratory mucus glycoproteins. *Infect Immun* 62, 1896-1900 (1994)

7. K.C. Kim. Biochemistry and pharmacology of mucin-like glycoproteins produced by cultured airway epithelial cells. *Exp Lung Res* 17, 533-545 (1991)

8. K.C. Kim, K. Wasano, R.M. Niles, J.E. Schuster, P.J. Stone and J.S. Brody JS. Human neutrophil elastase releases cell surface mucins from primary cultures of hamster tracheal epithelial cells. *Proc Natl Acad Sci USA* 84, 9304 9308 (1987)

9. S.J. Gendler, C.A. Lancaster, J. Taylor-Papadimitriou, T. Duhig, N. Peat, J. Burchell, L. Pemberton, E.N. Lalani and D. Wilson. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J Biol Chem* 265:15286-15293 (1990)

10. H.R. Park, S.W. Hyun and K.C. Kim. Expression of Muc1 mucin gene by confluent hamster tracheal epithelial cells in primary culture. *Am J Respir Cell Mol Biol* 15, 237-244 (1996)

11. E. Paul, D.I. Lee, S.W. Hyun, S. Gendler and K.C. Kim. Identification and characterization of high-molecular-mass mucin-like glycoproteins in the plasma membrane of airway epithelial cells. *Am J Respir Cell Mol Biol* 19, 681-690 (1998)

12. M. Rose and J. Voynow. Respiratory tract mucin genes and mucin glycoproteins in health and disease. *Physiol Rev* 86, 245-278 (2006)

13. J.A. Jarrard, R.I. Linnoila, H. Lee, S.M. Steinberg, H. Witschi and E. Szabo. MUC1 is a novel marker for the type II pneumocyte lineage during lung carcinogenesis. *Cancer Res* 58, 5582-5589 (1998)

14. C.L. Hattrup and S.J. Gendler. Structure and function of the cell surface (tethered) mucins. *Annu Rev Physiol* 70, 431-457 (2008)

15. K.C. Kim, S.W. Hyun, B.T. Kim, D. Meerzaman, M.K. Lee and E.P. Lillehoj. *Pseudomonas* adhesion to MUC1 mucins: A potential role of MUC1 mucins in clearance of inhaled bacteria. *Cilia, Mucus and Mucociliary Interactions*. (M. Salathe, ed.) Marcel Dekker, New York, pp. 217-224 (2001)

16. E.P. Lillehoj, S.W. Hyun, B.T. Kim, X.G. Zhang, D.I. Lee, S. Rowland and K.C. Kim. Muc1 mucins on the cell surface are adhesion sites for *Pseudomonas aeruginosa*. *Am J Physiol Lung Cell Mol Physiol* 280, L181-L187 (2001)

17. M. Bäckström, T. Link, F.J. Olson, H. Karlsson, R. Graham, G. Picco, J. Burchell, J. Taylor-Papadimitriou, T. Noll and G. Hansson. Recombinant MUC1 mucin with a breast cancer-like O-glycosylation produced in large amounts in Chinese-hamster ovary cells. *Biochem J* 376, 677-686 (2003)

18. E.P. Lillehoj, H. Kim, E.Y. Chun and K.C. Kim. *Pseudomonas aeruginosa* stimulates phosphorylation of the airway epithelial membrane glycoprotein Muc1 and activates MAP kinase. *Am J Physiol Lung Cell Mol Physiol* 287, L809-L815 (2004)

19. E.P. Lillehoj, W. Lu, T. Kiser, S.E. Goldblum and K.C. Kim. MUC1 inhibits cell proliferation by a beta-catenin-dependent mechanism. *Biochim Biophys Acta* 1773, 1028-1038 (2007)

20. D.J. Giard, S.A. Aaronson, G.J. Todaro, P. Arnstein, J.H. Kersey, H. Dosik and W.P. Parks. *In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J Natl Cancer Inst* 51, 1417-1423 (1973)

21. J. Zabner, P. Karp, M. Seiler, S.L. Phillips, C.J. Mitchell, M. Saavedra, M. Welsh and A.J. Klingelhutz. Development of cystic fibrosis and noncystic fibrosis airway cell lines. *Am J Physiol Lung Cell Mol Physiol* 284, L844-L854 (2003)

22. J. Ren, N. Agata, D. Chen, Y. Li, W.H. Yu, L. Huang, D. Raina, W. Chen, S. Kharbanda and D. Kufe. Human MUC1 carcinoma-associated protein confers resistance to genotoxic anticancer agents. *Cancer Cell* 5, 163-175 (2004)

23. I. Kuwahara, E.P. Lillehoj, T. Koga, Y. Isohama, T. Miyata and K.C. Kim. The signaling pathway involved in neutrophil elastase stimulated MUC1 transcription. *Am J Respir Cell Mol Biol* 37, 691-698 (2007)

24. M.A. Lindorfer, A. Nardin, P.L. Foley, M.D. Solga, A.J. Bankovich, E.N. Martin, A.L. Henderson, C.W. Price, E. Gyimesi, C.P. Wozencraft, J.B. Goldberg, W.M. Sutherland and R.P. Taylor. Targeting of *Pseudomonas aeruginosa* in the bloodstream with bispecific monoclonal antibodies. *J Immunol* 167, 2240-2249 (2001)

25. H. Wang, E.P. Lillehoj and K.C. Kim. Identification of four sites of stimulated tyrosine phosphorylation in the MUC1 cytoplasmic tail. *Biochem Biophys Res Commun* 310, 341-346 (2003)

26. H. Wang, E.P. Lillehoj and K.C. Kim. MUC1 tyrosine phosphorylation activates the extracellular signal-regulated kinase. *Biochem Biophys Res Commun* 321, 448-454 (2004)

27. L. Saiman and A. Prince A (1993) *Pseudomonas aeruginosa* pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells. *J Clin Invest* 92, 1875-1890.

28. L. Imundo, J. Barasch, A. Prince and Q. Al-Awqati. Cystic fibrosis epithelial cells have a receptor for pathogenic bacteria on their apical surface. *Proc Natl Acad Sci USA* 92, 3019-3023 (1995)

29. D.W. Martin, M.J. Schurr, M.H. Mudd, J.R. Govan, B.W. Holloway and V. Deretic. Mechanism of conversion to mucoidy in *Pseudomonas aeruginosa* infecting cystic fibrosis patients. *Proc Natl Acad Sci USA* 90, 8377-8381 (1993)

30. J.R. Govan and G.S. Harris. *Pseudomonas aeruginosa* and cystic fibrosis: unusual bacterial adaptation and pathogenesis. *Microbiol Sci* 3, 302-308 (1986)

31. A.J. Ratner, R. Bryan, A. Weber, S. Nguyen, D. Barnes, A. Pitt, S. Gelber, A. Cheung and A. Prince. Cystic fibrosis pathogens activate Ca^{2+}-dependent mitogen-activated protein kinase signaling pathways in airway epithelial cells. *J Biol Chem* 276, 19267-19275 (2001)

32. A.K. Dosanjh, D. Elashoff and R.C. Robbins. The bronchoalveolar lavage fluid of cystic fibrosis lung transplant recipients demonstrates increased interleukin-8 and elastase and decreased IL-10. *J Interferon Cytokine Res* 18, 851-854 (1998)

33. O. Tabary, S. Escotte, J.P. Couetil, D. Hubert, D. Dussie, E. Puchelle and J. Jacquot. High susceptibility for cystic fibrosis human airway gland cells to produce IL-8 through the IkappaB kinase alpha pathway in response to extracellular NaCl content. *J Immunol* 164, 3377-3384 (2000)

34. E. DiMango, H.B. Zar, R. Bryan and A. Prince. Diverse *Pseudomonas aeruginosa* gene products stimulate respiratory epithelial cells to produce interleukin-8. *J Clin Invest* 96, 2204-2210 (1995)

35. S.N. Abraham, B.L. Bishop, N. Sharon and I. Ofek. Adhesion of bacteria to mucosal surfaces. *Mucosal Immunology*. (P.L. Ogra, J. Mestecky, M.E. Lamm, W. Strober, J. Bienenstock and J.R. McGhee, eds.) Academic Press. San Diego, pp. 35-48 (2005)

36. R. Adamo, S. Sokol, G. Soong, M.I. Gomez and A. Prince. *Pseudomonas aeruginosa* flagella activate airway epithelial cells through asialo-GM1 and Toll-like receptor 2 as well as Toll-like receptor 5. *Am J Respir Cell Mol Biol* 30, 627-634 (2004)

37. K. Ogushi, A. Wada, T. Niidome, T. Okuda, R. Llanes, M. Nakayama, Y. Nishi, H. Kurazono, K.D. Smith, A. Aderem, J. Moss and T. Hirayama. Gangliosides act as co-receptors for *Salmonella enteritidis* FliC and promote FliC induction of human beta-defensin-2 expression in Caco-2 cells. *J Biol Chem* 279, 12213-12219 (2004)

38. A.P. West, B.A. Dancho and S.B. Mizel. Gangliosides inhibit flagellin signaling in the absence of an effect on flagellin binding to toll-like receptor 5. *J Biol Chem* 280, 9482-9488 (2005)

39. K. Oshikawa and Y. Sugiyama. Gene expression of Toll-like receptors and associated molecules induced by inflammatory stimuli in the primary alveolar macrophage. *Biochem Biophys Res Commun* 305, 649-655 (2003)

40. T.R. Hawn, A. Verbon, K.D. Lettinga, L.P. Zhao, S.S. Li, R.J. Laws, S.J. Skerrett, B. Beutler, L. Schroeder, A. Nachman, A. Ozinsky, K.D. Smith and A. Aderem. A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to Legionnaires' disease. *J Exp Med* 198, 1563-1572 (2003)

41. A. Muir, G. Soong, S. Sokol, B. Reddy, M.I. Gomez, A. van Heeckeren and A. Prince. Toll-like receptors in normal and cystic fibrosis airway epithelial cells. *Am J Respir Cell Mol Biol* 30, 777-783 (2004)

42. G.M. Barton and R. Medzhitov. Toll-like receptor signaling pathways. *Science* 300, 1524-1525 (2003)

43. A.T. Gewirtz, T.A Navas, S. Lyons, P.J. Godowski and J.L. Madara. Cutting edge: Bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J Immunol* 167, 1882-1885 (2001)

44. Y. Yu, H. Zeng, S. Lyons, A. Carlson, D. Merlin, A.S. Neish and A.T. Gewirtz. TLR5-mediated activation of p38 MAPK regulates epithelial IL-8 expression via posttranscriptional mechanism. *Am J Physiol Gastrointest Liver Physiol* 285, 282-290 (2003)

45. S.H. Rhee, A.C Keates, M.P. Moyer and C. Pothoulakis. MEK is a key modulator for TLR5-induced interleukin-8 and MIP3 gene expression in non-transformed human colonic epithelial cells. *J Biol Chem* 279, 25179-25188 (2004)

46. K. Ueno, T. Koga, K. Kato, D.T. Golenbock, S.J. Gendler, H. Kai and K.C. Kim. MUC1 mucin is a negative regulator of toll-like receptor signaling. *Am J Respir Cell Mol Biol* 38, 263-268 (2008)

47. E.L. Lagow and D.D Carson. Synergistic stimulation of MUC1 expression in normal breast epithelia and breast cancer cells by interferon-gamma and tumor necrosis factor-alpha. *J Cell Biochem* 86, 759-772 (2002)

48. S. Li, L. Wang, D.P. Nunes, R.F. Troxler and G.D. Offner. Pro-inflammatory cytokines up-regulate MUC1 gene expression in oral epithelial cells. *J Dent Res* 82, 883-887 (2003)

49. E.P. Lillehoj, F. Han and K.C. Kim. Mutagenesis of a Gly-Ser cleavage site in MUC1 inhibits ectodomain shedding. *Biochem Biophys Res Commun* 307, 743-749 (2003)

Key Words: Mucin, Toll-Like Receptor, Innate Immunity, Cystic Fibrosis

Send correspondence to: Kwang Chul Kim, Department of Physiology and Lung Center, Temple University School of Medicine, MRB Room 411, 3420 N. Broad Street, Philadelphia, PA 19140, Tel: 215-707-9084, Fax: 215-707-0170, E-mail: kckim@temple.edu

http://www.bioscience.org/current/vol2E.htm