Deglycosylated MUC1 Ectodomain for the Diagnosis and Treatment of *Pseudomonas aeruginosa* Lung Infections

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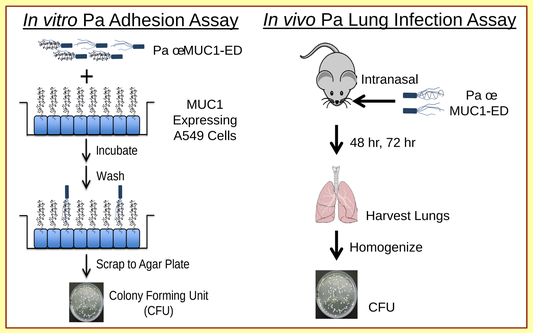
ABSTRACT

Background: *Pseudomonas aeruginosa* (Pa) is a major opportunistic pathogen of human airways, but the host response to infection is incompletely understood. Epithelial cells lining the airways express numerous surface receptors that recognize infectious agents such as Pa. One such receptor, MUC1, recognizes Pa flagellin, the major structural protein of the bacterial flagellum. MUC1 consists of an NH_2-terminal, highly O-glycosylated ectodomain (MUC1-ED) attached to the cell surface through a membrane-spanning domain. MUC1-ED is proteolytically processed and shed from the epithelial cell surface following cleavage at a juxtamembranous Gly-Ser peptide bond. We previously demonstrated that stimulation of human airway epithelial cells with Pa flagellin increased MUC1-ED shedding *in vitro* (Lillehoj *et al*., J. Biol. Chem. 290:18316, 2015). Here, we asked whether MUC1-ED is also shed *in vivo*, and if so, whether shed MUC1-ED might serve as a diagnostic biomarker of Pa infection. Further, we prepared MUC1-ED isoforms that were either fully glycosylated, partially deglycosylated through desialylation, completely O-deglycosylated with retention of N-glycosylation, or combined N- and O-deglycoyslated, and screened these glycoforms for inhibition of Pa adhesion to human airway epithelial cells *in vitro* and Pa lung infection using an *in vivo* mouse model.

Results: Intranasal administration of Pa bacteria or its purified flagellin to mice increased desialylated MUC1-ED levels in bronchoalveolar lavage fluid (BALF) compared with PBS controls. Shed human MUC1-ED levels were greater in BALF from Pa-colonized patients compared with noncolonized individuals or patients colonized with non-Pa bacteria. BALF from Pa-colonized patients inhibited Pa adhesion to A549 cells compared with BALF from noncolonized subjects or patients colonized with non-Pa bacteria. MUC1-ED immunodepletion of BALF from Pa-colonized patients restored their inhibitory activity for Pa adhesion. Incubation of Pa with desialylated MUC1-ED decreased Pa adhesion to A549 cells. Incubation of Pa with O-deglycosylated MUC1-ED, or with combined N- and O-deglycosylated MUC1-ED, further reduced Pa adhesion compared with desialylated MUC1-ED. Incubation of Pa with N- and O-deglycosylated MUC1-ED reduced the number of Pa recovered from the BALF of mice following *in vivo* infection.

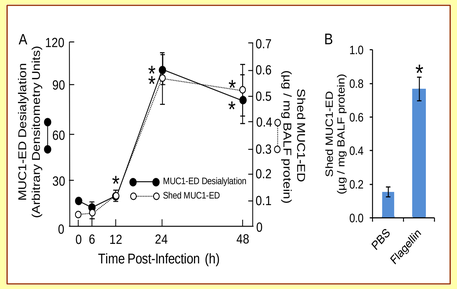
Conclusions: These results suggest that shed MUC1-ED in human BALF might serve as a diagnostic biomarker for Pa lung infection, and that deglycosylated MUC1-ED, or synthetic peptides derived from the MUC1-ED protein backbone, may offer a novel therapeutic intervention for invasive Pa lung infections.

METHODS



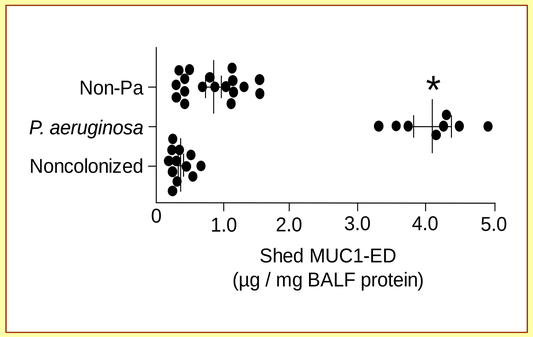
RESULTS

Figure 1. *Pseudomonas aeruginosa* and Flagellin Increase MUC1-ED Shedding *In Vivo*.



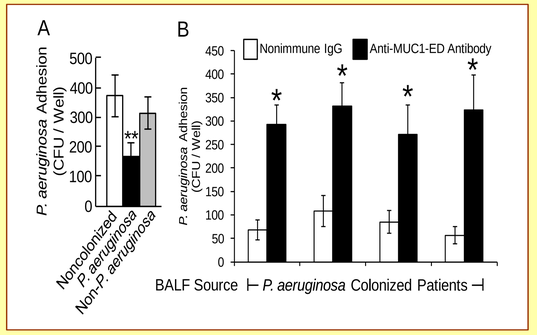
Mice were intranasally challenged with (A, B) PBS, (A) 1x10^5 CFU/mouse of Pa bacteria, or (B) 100 ng/mouse of Pa-derived flagellin. BALF was collected at (A) increasing times or (B) 24 hr postchallenge. (A, left ordinate) Desialylation of MUC1-ED was assessed by quantitative PNA lectin blotting. (A, right ordinate; B) MUC1-ED levels were quantified by ELISA. Data points/bars represent mean ± SEM values (n=3). *, increased MUC1-ED desialylation or shedding *vs*. PBS controls at p<0.05.

Figure 2. Shed MUC1-ED Levels are Greater in BALF of Patients with *Pseudomonas aeruginosa* Pneumonia.



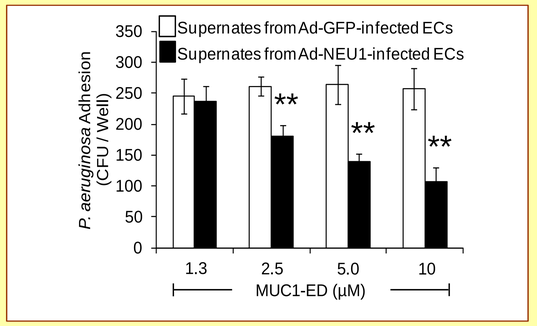
MUC1-ED levels in BALF from noncolonized patients, Pa-colonized patients, or patients colonized with non-Pa microorganisms were quantified by ELISA and normalized to total BALF protein. *, increased mean MUC1-ED levels in BALF from Pa-colonized patients *vs*. levels in BALF from noncolonized patients or patients colonized with non-Pa microbes at p<0.05.

Figure 3. MUC1-ED in BALF of *Pseudomonas aeruginosa*-Colonized Patients Inhibits Bacterial Adhesion to Airway Epithelial Cells.



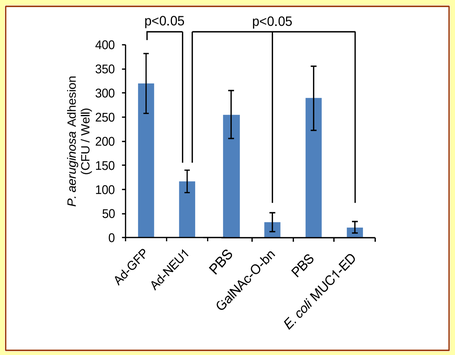
(A) Pa were incubated with BALF from Pa-colonized patients, noncolonized patients, or patients colonized with non-Pa microorganisms, and the bacteria were assayed for adhesion to A549 cells. **, decreased Pa adhesion *vs.* noncolonized or non-Pa-colonized patients at p<0.05. (B) BALFs from Pa-colonized patients were immunodepleted with anti-MUC1-ED antibody, or incubated with a nonimmune IgG control. Pa were incubated with the BALFs and assayed for adhesion to A549 cells. Bars represent mean ± SEM CFUs/well (n=6). *, increased Pa adhesion *vs*. nonimmune IgG incubation at p<0.05.

Figure 4. Desialylated MUC1-ED Inhibits *Pseudomonas aeruginosa* Adhesion to Airway Epithelial Cells.



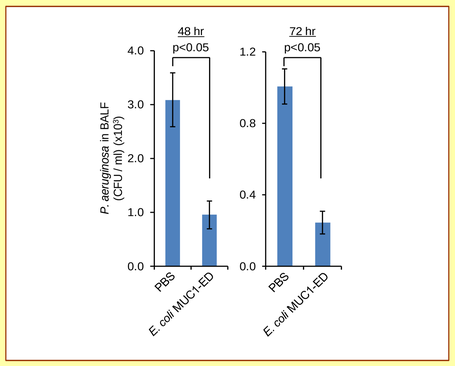
A549 cells infected with adenovirus encoding NEU1 sialidase (Ad-NEU1) or Ad-GFP control and shed, desialylated MUC1-ED levels in culture supernatants were quantified by ELISA. Pa were incubated with the culture supernatants containing increasing amounts of MUC1-ED, or an equivalent volume of control supernatants, and assayed for adhesion to fresh A549 cell monolayers. **, decreased Pa adhesion at p<0.05. Bars represent mean œSEM CFUs/well (n=3).

Figure 5. Deglycosylated MUC1-ED Inhibits *Pseudomonas aeruginosa* Adhesion Greater than Desialylated MUC1-ED.



Shed, desialylated MUC1-ED was prepared from A549 cells infected with Ad-NEU1 or Ad-GFP control. O-deglycosylated MUC1-ED was prepared from A549 cells incubated in the presence of GalNAc-O-bn. Combined N- and O-deglycosylated MUC1-ED was synthesized in *E. coli*. Pa were incubated with the different MUC1-ED glycosylation isoforms and assayed for adhesion to A549 cells. Bars represent mean œSEM CFUs/well (n=3).

Figure 6. *E. coli*-derived MUC1-ED Reduces *Pseudomonas aeruginosa* Lung Infection *In Vivo*.



Pa were incubated with PBS or combined N- and O-deglycosylated MUC1-ED expressed in *E. coli*. Mice were intranasally challenged with the bacteria and colony forming units (CFUs) in bronchoalveolar lavage fluid (BALF) were measured at 48 hr and 72 hr post-infection. Bars represent mean œSEM CFUs/ml BALF (n=3).