Neuraminidase-1 Desialylates the MUC1 Ectodomain to Release a Decoy Receptor that Protects against Lethal *Pseudomonas aeruginosa* Lung Infection

Erik P. Lillehoj,[1] Wei Guang,[1] Sang W. Hyun,[2,3] Anguo Liu,[2,3] Nicolas Hegerle,[2,4] Raphael Simon,[2,4] Alan S. Cross,[2,4] Hideharu Ishida,[5] Irina G. Luzina,[2,3] Sergei P. Atamas,[2,3] and Simeon E. Goldblum[2,3]

[1]Department of Pediatrics, [2]Department of Medicine, and [4]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD; [3]U.S. Department of Veterans Affairs, Baltimore VA Medical Center, Baltimore, MD; Department of Applied Bio-organic Chemistry, Gifu University, Gifu, Japan

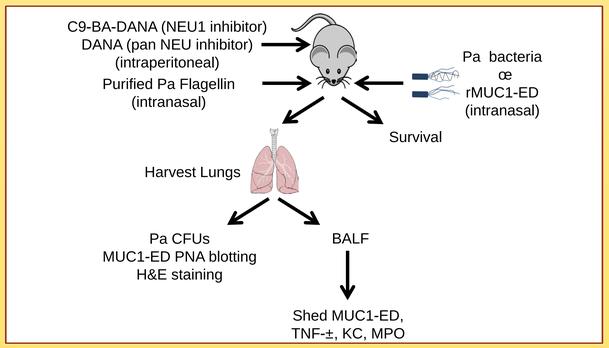
ABSTRACT

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). Using an intact, physiologically relevant murine model of Pa pneumonia, here we asked whether Pa and its flagellin might also stimulate NEU1-dependent MUC1-ED desialylation *in vivo* to release a hyperadhesive decoy receptor that provides a novel, protective host response to Pa lung infection.

Results: Intranasal administration of e5.0x10^3 colony forming units of Pa strain K (PAK) to BALB/c mice increased MUC1-ED shedding into the bronchoalveolar compartment. MUC1-ED levels increased as early as 12 h, peaked at 24-48 h, reaching up to a 7.8-fold increase, and decreased by 72 h. The a-type flagellin-expressing PAK strain and the b-type flagellin-expressing PAO1 strain stimulated comparable levels of MUC1-ED shedding. A flagellin-deficient isogenic PAK mutant provoked dramatically reduced MUC1-ED shedding compared with the wild-type strain, and purified flagellin recapitulated the wild-type effect. In lung tissues, Pa increased MUC1-ED desialylation by peanut agglutinin lectin blotting. NEU1-selective sialidase inhibition with C9-BA-DANA, or use of a catalytically-inactive NEU1-G68V mutant, protected against Pa-induced MUC1-ED desialylation and shedding. MUC1-ED inhibition of *in vitro* Pa adhesion and flagellin binding to airway epithelial cells was localized to its protein backbone and not to its glycans. Finally, co-administration of Pa with human recombinant (r)MUC1-ED expressed in *E. coli* diminished lung and BALF bacterial burden, proinflammatory cytokine levels, and pulmonary leukostasis, and enhanced 5-day survival from 0% to 75%.

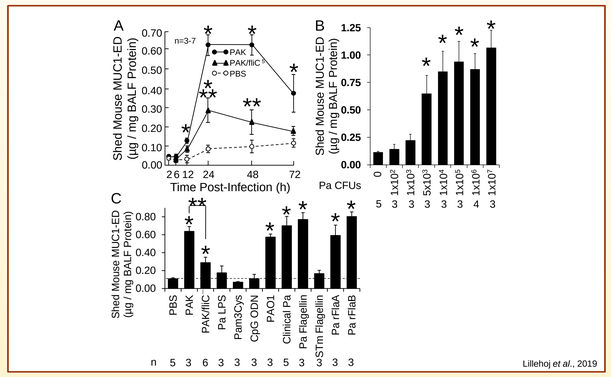
Conclusions: These combined data indicate that Pa flagellin provokes NEU1-mediated airway shedding of MUC1-ED as a decoy receptor that protects against lethal Pa lung infection. Human rMUC1-ED might someday be harnessed as a therapeutic intervention to target Pa lung infections, including those associated with multi-drug resistant organisms.

METHODS



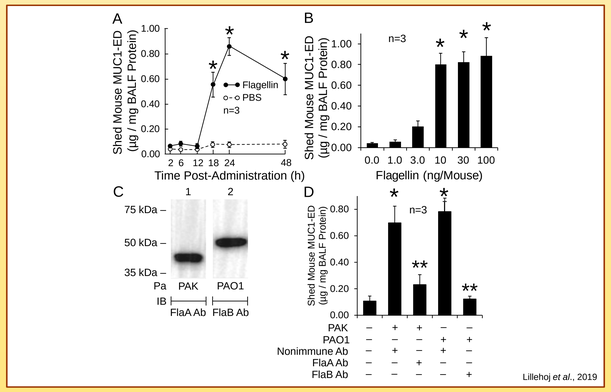
RESULTS

Figure 1. Flagellin-expressing Pa induces MUC1-ED shedding *in vivo.*



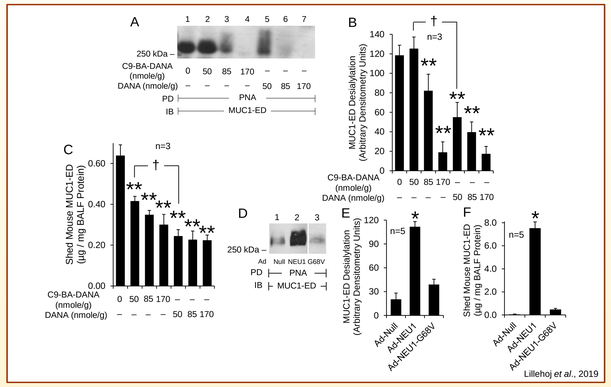
(A) BALB/c mice were administered intranasally (i.n.) with 1.0x10^5 CFUs/mouse of WT PAK, the flagellin-deficient PAK/fliC• mutant, or PBS and MUC1-ED levels in BALF quantified by ELISA. (B) At 24 h post-infection with increasing inocula of WT PAK, MUC1-ED levels in BALF were quantified by ELISA. (C) Mice were administered i.n. with 1x10^5 CFUs of the indicated strains of Pa, 10 ng of Pa or STm flagellins, 10 ng of Pa rFlaA or rFlaB flagellins, 100 ng of Pa LPS, 10 µg of Pam_3Cys-Ser-(Lys)4, or 10 µg of CpG ODN 1826. At 24 h post-administration, MUC1-ED levels in BALF were quantified.

Figure 2. Purified Pa flagellin recapitulates Pa-provoked MUC1-ED shedding *in vivo*.



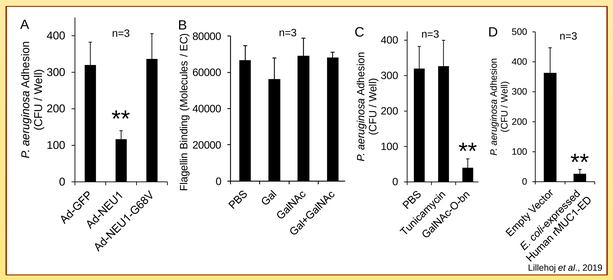
(A) BALB/c mice were administered i.n. with 10 ng/mouse of PAK flagellin or PBS and MUC1-ED levels in BALF quantified by ELISA. (B) Mice were administered increasing doses of PAK flagellin. At 24 h post-treatment, MUC1-ED levels in BALF were quantified by ELISA. (C) Validation of mouse anti-FlaA and anti-FlaB antisera using lysates of PAK (a-type) and PAO1 (b-type). (D) PAK bacteria were preincubated with anti-FlaA antiserum or nonimmune mouse serum, and PAO1 bacteria were preincubated with of anti-FlaB antiserum or nonimmune mouse serum and washed. BALB/c mice were administered i.n. with the bacteria or PBS control, and MUC1-ED levels in BALF quantified at 24 h post-administration.

Figure 3. NEU1 is required for Pa-induced MUC1-ED desialylation and shedding.



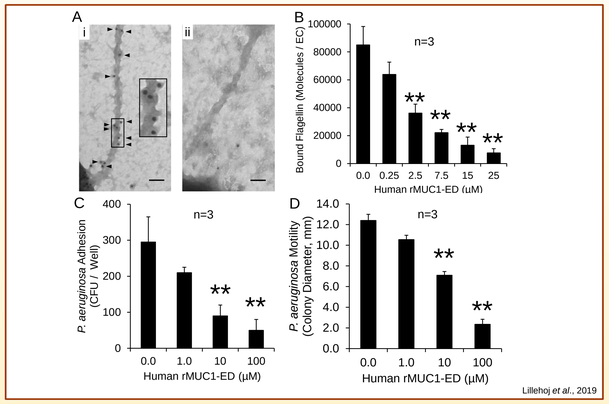
(A) BALB/c mice were administered i.p. with the NEU1 selective inhibitor C9-BA-DANA, DANA, or PBS. At 24 h post-administration, mice were infected i.n. with 1.0x10^5 CFUs/mouse of Pa. At 24 h post-infection, BALFs were incubated with PNA-agarose and the PNA-binding proteins processed for MUC1-ED immunoblotting. (B) Densitometric analysis. (C) BALFs were processed for MUC1-ED levels by ELISA. (D) BALB/c mice were administered i.t. with Ad-NEU1, Ad-NEU1-G68V, or Ad-Null. At 3 days post-infection, BALFs were incubated with PNA-agarose and the PNA-binding proteins processed for MUC1-ED immunoblotting. (E) Densitometric analysis. (F) BALFs were processed for MUC1-ED levels by ELISA.

Figure 4. The deglycosylated protein backbone of human MUC1-ED inhibits Pa adhesion to human airway epithelial cells.



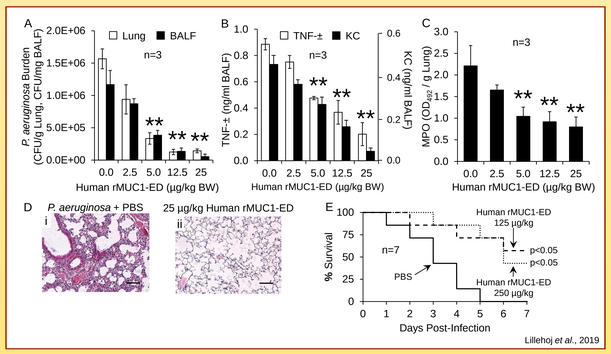
(A) Pa bacteria were incubated with human MUC1-ED isolated from supernatants of A549 airway epithelial cells infected with Ad-NEU1, Ad-NEU1-G68V,or Ad-GFP, washed, and assayed for adhesion to fresh, unmanipulated A549 cells. (B) Alexa Fluor 594-labeled Pa flagellin was incubated with 25 mM of Gal or GalNAc, 25 mM Gal plus 25 mM GalNAc, or PBS. The cells were washed and processed for flagellin binding by fluorometry. (C) Pa was incubated with human MUC1-ED isolated from the supernatants of A549 cells cultured in the presence of 1.0 µg/ml tunicamycin, 5.0 µM GalNAc-O-bn, or PBS, washed, and assayed for adhesion to fresh, unmanipulated A549 cells. (D) Pa was incubated with human recombinant (r)MUC1-ED prepared from *E. coli* transformed with a MUC1 expression plasmid or *E. coli* transformed with the empty vector control, washed, and assayed for adhesion to A549 cells.

Figure 5. Human recombinant MUC1-ED inhibits flagellin binding and Pa adhesion to human airway cells and Pa motility.



(A) Pa was incubated with 25 µM of human rMUC1-ED, the bacteria were washed and incubated with mouse anti-MUC1-ED Ab or nonimmune mouse IgG, followed by gold-labeled goat anti-mouse IgG secondary Ab, and examined by transmission immunoelectron microscopy. (B, C) Human rMUC1-ED or PBS were incubated with Alexa Fluor 594-labeled Pa flagellin or Pa and assayed for (B) flagellin binding or (C) Pa adhesion to Ad-NEU1-infected A549 cells. (D) Human rMUC1-ED or PBS were incubated with Pa and the bacteria processed for motility.

Figure 6. Human recombinant MUC1-ED protects against lethal Pa lung infection.



(A-C) Pa (1.0x10^5 CFUs/mouse) were co-administered i.n. with increasing concentrations of human rMUC1-ED or PBS to BALB/c mice. (A) Pa CFUs in lung and BALF, (B) TNF-± and KC levels in BALF, and (C) myeloperoxidase (MPO) activity in lung as a biochemical marker of pulmonary leukostasis were measured at 24 h post-administration. (D) Pa (1.0x10^5 CFUs/mouse) was co-administered i.n. with 25 µg/kg of human rMUC1-ED or PBS to BALB/c mice. At 24 h post-administration, lung sections were stained with hematoxylin and eosin, and examined by microscopy. Scale bar, 50 µM. (E) Pa (2.0x10^7 CFUs/mouse) was co-administered i.n. with 125 or 250 µg/kg of human rMUC1-ED or PBS to BALB/c mice and survival was measured daily by Kaplan-Meier analysis.

Supported by NIH grant 1R21-AI144497-01 and a University of Maryland Baltimore Institute for Clinical and Translational Research (ICTR) Accelerated Translational Incubator Pilot (ATIP) award (EPL), and VA grant I01BX002352 (SEG).