

Pseudomonas aeruginosa Flagellin Regulates NEU1 Sialidase-Responsive, MUC1 Ectodomain-Dependent Bacterial Adhesion to and Invasion into Human Airway Epithelial Cells

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Abstract

Background: *Pseudomonas aeruginosa* (Pa) is a major opportunistic pathogen of human airways, but its pathogenic mechanisms are incompletely understood. Epithelial cell (EC)s lining the airways express numerous surface receptors that recognize infectious pathogens such as Pa. One such airway EC receptor is MUC1, a heterodimeric complex through an NH₂-terminal, highly sialylated ectodomain (MUC1-ED) noncovalently associated with a membrane-tethered subunit containing a cytoplasmic domain (MUC1-CD). Pa flagellin, the major structural protein of the bacterial flagellum, is a ligand for MUC1-ED. As with all sialoproteins, the sialylation state of MUC1-ED is dynamically and coordinately regulated through the opposing catalytic activities of sialyltransferases and neuraminidase/sialidase (NEU)s. Our prior study demonstrated that of the four known mammalian NEUs, human airway ECs predominantly express NEU1, and that NEU1 overexpression increased Pa adhesion to these ECs (Lillehoj et al., *J. Biol. Chem.* 287:8214, 2012).

Results: We asked whether NEU1 might also increase adhesion of airway bacterial pathogens other than Pa. NEU1 overexpression in human airway ECs increased adhesion of *Legionella pneumophila*, but not of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, or *Klebsiella pneumoniae*. This NEU1-responsive adhesion was MUC1-dependent for Pa, but not for *L. pneumophila*. Pa expression of flagellin was required for NEU1-responsive Pa adhesion to airway ECs. NEU1 expression increased the binding affinity of flagellin for airway ECs approximately 3.0-fold. Flagellin treatment of airway ECs stimulated NEU1 recruitment to MUC1. Glutathione S-transferase (GST)-NEU1 binding assays established direct NEU1 association with MUC1-CD, but not with MUC1-ED. Treatment of airway ECs with flagellin increased NEU1-dependent desialylation of MUC1-ED, and amplified NEU1-dependent Pa adhesion to and invasion into airway ECs. These combined results indicate that Pa utilizes a flagellin-dependent mechanism to recruit human NEU1 to the MUC1-CD, thereby desialylating the MUC1-ED, to promote Pa adhesion and invasion.

Acknowledgments

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Figure 1. NEU1 Selectively Enhances MUC1-Dependent *P. aeruginosa* Adhesion to Airway ECs.

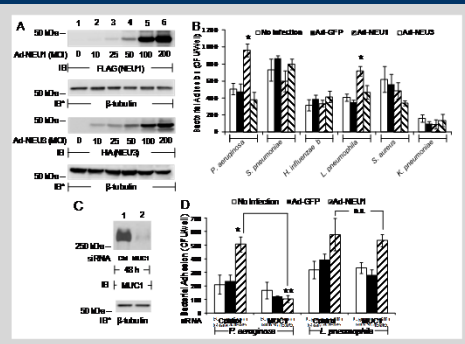


Figure 2. NEU1 Regulates Flagellin-Dependent Pa Adhesion to MUC1-Expressing ECs.

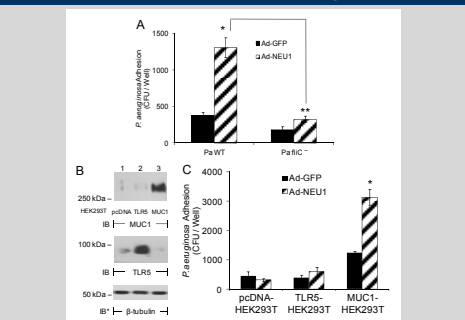


Figure 3. NEU1 Increases the Binding Affinity of Flagellin for MUC1-Expressing Airway ECs.

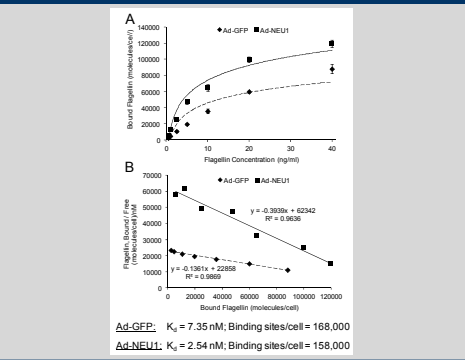


Figure 4. Flagellin Stimulates NEU1 Recruitment to MUC1.

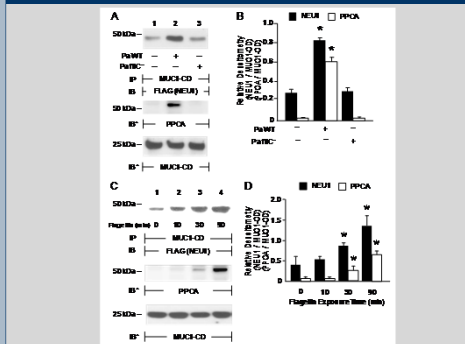


Figure 5. NEU1 Binds to the NH₂-Terminal Portion of MUC1-CD.

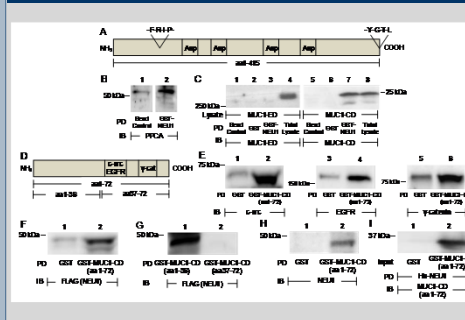


Figure 6. Flagellin Stimulates NEU1-Dependent MUC1-ED Desialylation.

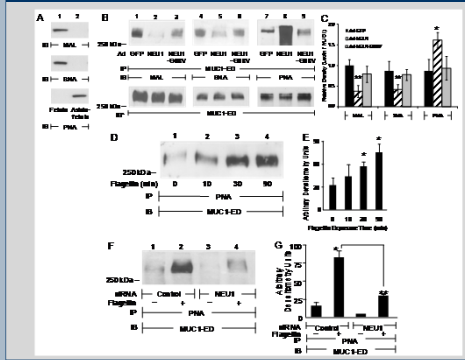


Figure 7. NEU1 is Required for Flagellin-Induced Increases in Pa Adhesion.

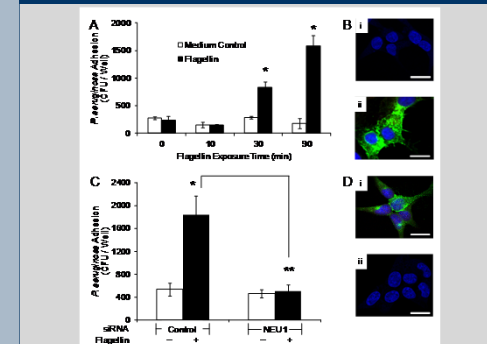


Figure 8. Flagellin Stimulates NEU1-Regulated, MUC1-Dependent Pa Internalization into Airway ECs.

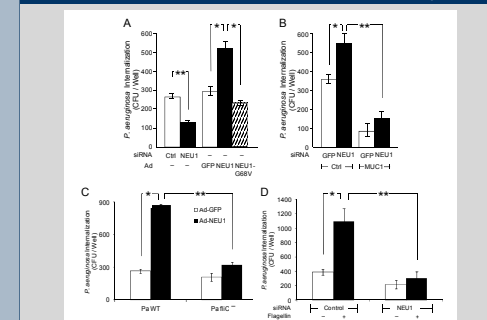


Figure 9. Hypothetical Model

