NEU1 Sialidase Regulates MUC1-Dependent Bacterial Adhesion to and Invasion of Human Airway Epithelia

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Abstract

Pseudomonas aeruginosa (Pa) is an important airway opportunistic pathogen in a variety of clinical settings, including cystic fibrosis and the necrotizing pneumonia presenting in individuals with neutropenia, alcoholism, and on mechanical ventilation. Epithelial cell (EC)s lining the airways express numerous surface receptors that recognize infectious pathogens such as Pa. MUC1 is one such airway EC receptor. MUC1 is a heterodimeric complex between an NH_2-terminal, O-glycosylated ectodomain noncovalently associated with a membrane-tethered subunit containing an intracellular domain. We have established Pa-derived flagellin, the major structural protein of the bacterial flagellum, as a ligand for the highly sialylated MUC1 ectodomain. The sialylation state of MUC1, as with all sialoproteins, is dynamically and coordinately regulated through the opposing catalytic activities of sialyltransferases and neuraminidase/sialidase (NEU)s. Of the four known mammalian NEUs, airway ECs predominantly express NEU1. The current studies were performed to test the hypothesis that Pa and Pa-deived flagellin stimulate the recruitment of NEU1 to the MUC1 cytoplasmic domain, that NEU1 association with the MUC1 cytoplasmic domain leads to desialylation of the MUC1 ectodomain, and that NEU1-mediated MUC1 ectodomain desialyation promotes Pa adhesion to and internalization into airway ECs. In co-immunoprecipitation assays, treatment of A549 airway ECs with Pa or its flagellin stimulated association of the MUC1 cytoplasmic domain with NEU1. *In vitro* binding assays mapped the amino acid (aa) sequences within the 72-aa MUC1 cytoplasmic domain required for NEU1 recruitment to its proximal half (aa1-36). In lectin blotting experiments, overexpression of NEU1 in A549 cells by infection with an adenovirus (Ad)-NEU1 construct decreased MUC1 ectodomain binding to *Maackia amurensis* lectin (MAL) II, which recognizes terminal sialic acid residues in ±-2,3-linkages, compared with cells infected with adenovirus alone (Ad-Null). In contrast, NEU1 overexpression increased MUC1 ectodomain binding to peanut agglutinin (PNA), which binds to subterminal galactose after removal of terminal Sia, compared with Ad-Null infection. Conversely, NEU1 knockdown by transfection with small interfering (si)RNA increased MUC1 ectodomain binding to MAL II, and decreased binding to PNA, compared with non-targeting control siRNA-transfected cells. Infection of A549 cells with Ad-NEU1 enhanced MUC1 ectodomain-dependent adhesiveness for Pa, compared with cells infected with Ad-Null, while NEU1 knockdown had the opposite effect. NEU1 overexpression also increased the binding of Pa-derived flagellin to these same cells. In a gentamicin protection assay, Ad-NEU1 infection of A549 cells increased Pa internalization into the cells, compared with Ad-Null, while NEU1 silencing reduced Pa internalization, compared with control siRNA-transfected cells. By double-label immunofluorescence microscopy, Pa was detected within intact, MUC1 ectodomain-expressing A549 cells. Finally, immunoblot analysis of purified, LAMP-1-expressing lysosomes identified both the MUC1 ectodomain and Pa.

Figure 1. NEU1 is recruited to the MUC1 cytoplamsic domain.

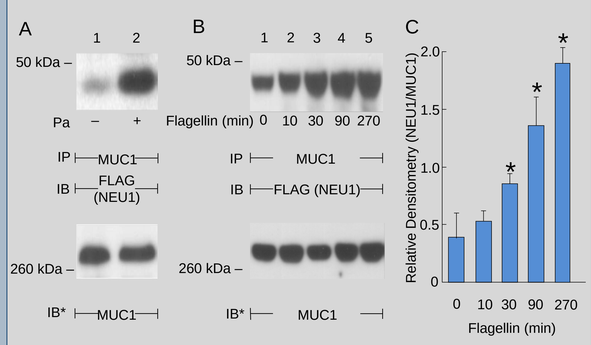


Figure 2. NEU1 is recruited to the proximal half of the MUC1 cytoplasmic domain.

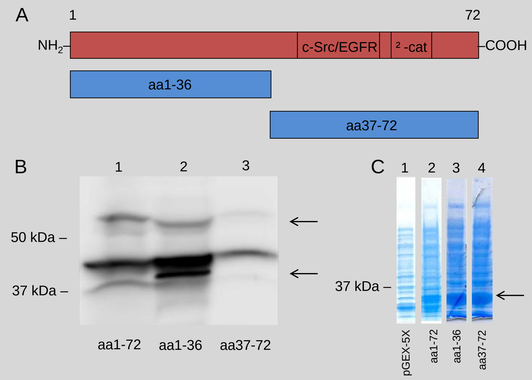


Figure 3. The MUC1 ectodomain is an *in vivo* substrate for NEU1.

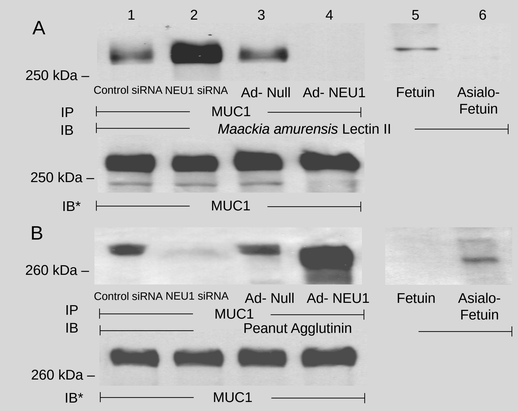


Figure 4. NEU1 regulates Pa adhesion to airway epithelial cells.

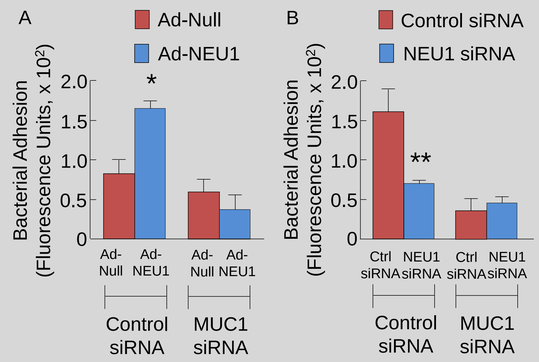


Figure 5. NEU1 regulates Pa flagellin binding to airway epithelial cells.

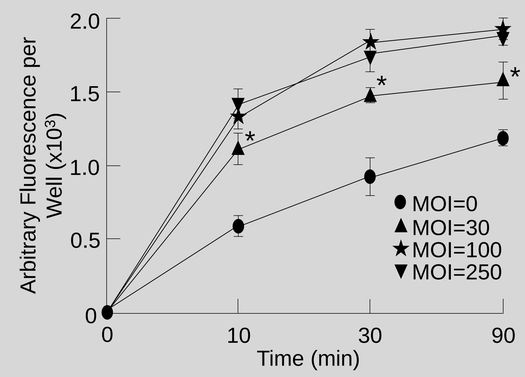


Figure 6. Pa is internalized into MUC1-expressing airway epithelial cells.

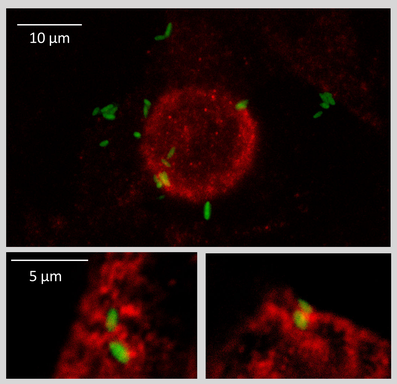


Figure 7. The MUC1 ectodomain and Pa are internalized into LAMP-1 lysosomes.

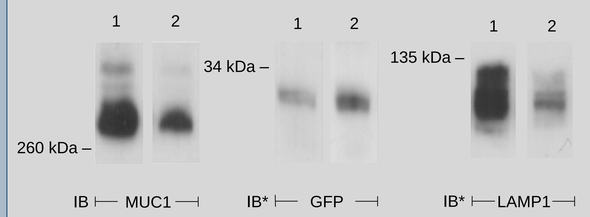
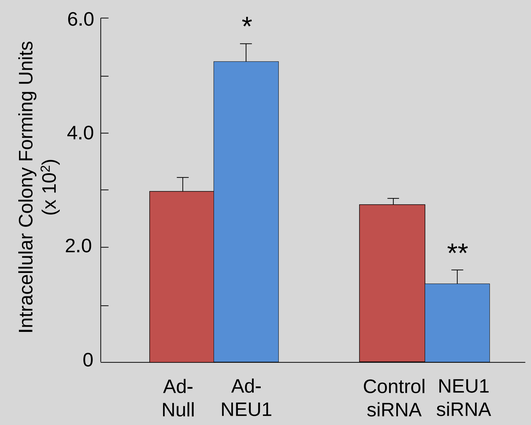


Figure 8. NEU1 regulates Pa internalization into airway epithelial cells.



Summary

1) Pa and its flagellin stimulate NEU1 recruitment to the MUC1 cytoplasmic domain.
2) NEU1 recruited to the MUC1 cytoplasmic domain desialylates the MUC1 ectodomain.
3) NEU1-mediated desialylation of the MUC1 ectodomain promotes Pa adhesion to and lysosomal internalization into human airway ECs.

Acknowledgments

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