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Grant Title: Galectin Inhibitors for Sepsis Prevention and Therapy

Abstract

1. Objectives: Our prior studies showed that galectins expressed and secreted by the airway epithelia contribute to the enhanced susceptibility of influenza patients to pneumococcal pneumonia and ensuing hypercytokinemia. Specifically, we showed that unmasking of galactosyl moieties by the influenza A virus neuraminidase on the airway epithelial surface that takes place upon a primary viral infection, leads to enhanced galectin binding and pneumococcal adhesion. Thus, we proposed that inhibiting the binding of galectins to the airway epithelia during IVA infection will prevent or ameliorate the pneumococcal superinfection and ensuing sepsis. To test this hypothesis, we conducted studies with the following objectives: (a) identify and characterize galectin receptors on the airway cell surface, and the effectiveness of novel high affinity/avidity multivalent inhibitors of galectin binding (MSIGB) in disrupting galectin-receptor interactions, and (b) assess the effectiveness of MSIGB for preventing a dysregulated cytokine response

2. Methods used: For the identification of galectin receptors on the airway epithelial cell surface, we used affinity chromatography of A549 cell extracts on immobilized human recombinant galectin 1 (Gal1) and galectin 3 (Gal3). The eluted galectin ligands were electrophoresed (PAGE), transferred to PVDF membranes, and developed with recombinant Gal1 and Gal3, and specific antibodies against selected cell surface receptors. The *in vitro* characterization of the specificity of the galectin-receptor interactions was assessed by ELISA, testing binding-inhibition with selected oligosaccharides and glycoproteins. The design, synthesis and validation of MSIGB, was carried out in collaboration with Dr. L-X. Wang (UMDCP). Well-established methods in Dr. Wang's lab were applied for the synthesis, analysis, and spectroscopic validation of the MSIGB that were based on a cyclodextrin (CD) core. Binding-inhibition specificity of MSIGB was measured by ELISA and affinity by surface plasmon resonance (SPR). The effectiveness of MSIGB in disrupting galectin-mediated downregulation of SOCS expression was carried by standard and qRT-PCR. The preventive/therapeutic value of the MSIGB in our mouse model for post-influenza pneumonia/sepsis is currently assessed in collaboration with Dr. A.S. Cross, UMB).

3. Results: The first group of experiments was focused on the identification of Gal3 receptors on the airway cell surface and revealed the presence of $\alpha 3\beta 1$ integrin and MUC1; ongoing studies are aimed at confirming the presence of CD147. The synthesis of inhibitors for Gal3 binding was successful in that both monovalent and CD- based multivalent inhibitors were synthesized, with non-reducing terminal galactosyl moieties, namely lactose (Lac), N-acetylgalactosamine (GalNAc), and the Thompsen-Friedenreich disaccharide (TFD; Galβ1,3GalNAc). The MSIGB were effective not only in preventing binding of Gal3 to desialylated glycoproteins such as asialofetuin, but also to airway epithelial cells (A549 cell line). Most importantly, the results showed that MSIGB are effective in hindering Gal3 binding. Further, our studies demonstrated that by preventing the binding of Gal3 to the airway cell surface the synthetic inhibitors were also effective in preventing the downregulation of SOCS, a key achievement for the prevention of the cytokine storm that results from the post-influenza pneumococcal pneumonia. Animal testing of the inhibitors' efficacy and lack of toxicity is ongoing.