Dendritic Cell Responses to Surfaces Presenting Defined Glycans

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Statement of Purpose: The goal of this research is to elucidate dendritic cell (DC) responses to defined glycan presenting surfaces. Specifically, these surfaces are modified to present glycans that are known to be associated with glycoproteins of serum or associated with pathogen surfaces. The impetus for this research is that DCs have been shown to respond to exogenous pathogens such as parasitic and bacterial glycan moieties as well as atypical glycosylation of cancerous and virally infected cells. It is hypothesized that the glycoform presented by adsorbed glycoproteins on implanted biomaterial surfaces influences cellular interactions, particularly those of inflammatory/immune cells, through their cognate C-type lectin receptors. Furthermore, glycans were detected using enzyme linked lectin assays (ELLAs) in the adsorbed serum layer on self-assembled monolayers of defined chemistries[1]. However, the contributions of specific glycans in the complex biomolecule adsorbate to DC responses were difficult to elucidate. Due to the complexity of glycan structures, and their constituent parts, a high throughput methodology is most amenable to analysis of resultant DC responses including marker expression and cytokine profiles. Lewis (Le) and mannose-containing glycans and their constituents were biotinylated and immobilized in wells of a streptavidin (SA)-coated 96-well plate for treatment of DCs.

Methods: Isolation of the high mannose structures from Ribonuclease B, RNase B (Worthington Biochemical) was performed via enzymatic cleaving using PNGase F. Mannose-containing glycans were isolated and functionalized with 2-amino-N-(2-amino-ethyl)-benzamide in 1M $NaCNBH_3$ (2 h, 65°C), followed by isolation and purification using HPLC. These structures were conjugated with biotin using NHS-LC-biotin (4 h). Biotinylated Le structures were kindly provided by the Consortium for Functional Glycomics (NIH/NHLBI). Control monosaccharides were obtained from Glycotech. Biotinylated glycans were captured on SA-coated surfaces prepared by pre-adsorbing SA [15 µg/mL in 0.1M $NaHCO_3$; 20 h; room temperature (rt)] in 96-well Costar TCPS plates and wells blocked with filter-sterilized 5 mg/mL BSA and 0.1 µg/mL SA in 0.1 M $NaHCO_3$ (2 h; rt). Biotinylated glycans (100 µl, 500-5000 pmol/well, 24 h) were immobilized in wells and amounts quantified using ELLAs. Preparation of these surfaces was optimized so as to minimize the background levels of DC maturation on the SA-coated surface and to ensure that a saturating concentration of glycan was being used when immobilizing the glycans in the plates based on detection using ELLAs. Immature DCs (iDCs) were derived from peripheral blood mononuclear cells in media containing GM-CSF and IL-4 for 5 days according to an established protocol.[2] iDCs were then treated with immobilized glycans in 96 well plates for 24 h. The extent of DC maturation was compared to controls: untreated iDCs and lipopolysaccharide-treated mature DCs (mDC) cultured on TCPS or SA-coated wells. All treated DCs and controls were transferred to a black 96-well filter plate and dual-stained with anti-CD86-PE and anti-DC-SIGN-FITC for detection using a fluorescent plate reader and determination of the maturation metric "CD86/DC-SIGN". Supernatants were collected and stored at -80°C for multiplex cytokine profiling.

Results: Optimization of the SA coating and blocking was performed in order to obtain a non-activating background surface as seen in (Fig. 1) and a saturating plating glycan concentration of ~4000 pmol/well was determined by ELLAs (Fig. 2). DCs treated with a library of Le and mannose-containing glycans and their constituents showed a differential DC phenotypic response as shown for the maturation metric "CD86/DC-SIGN" (Fig. 1), wherein Le^y-Le^x was particularly noteworthy in its stimulatory effect on DCs.

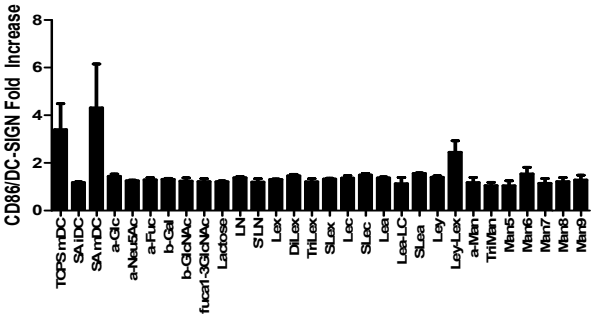

Figure 1: DC response to a library of Le- and mannose-containing glycans and their consistent parts as denoted by maturation metric (CD86/DC-SIGN fold increase over value for that for TCPS iDCs), average + range/2 for two donors; n=3 wells/donor.

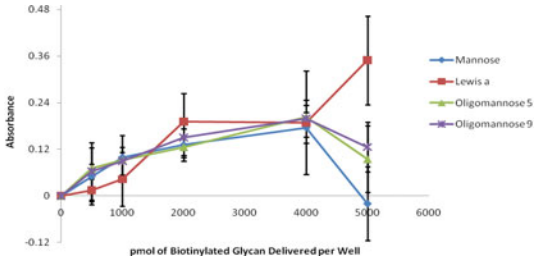

Figure 2: ELLA absorbance signal for mannose, Lewis[a], oligomannose 5 or oligomannose 9 as a function of glycan amount per 96-well; mean ± S.D., n=6 independent trials.

Conclusions: DCs can be treated with defined glycans immobilized on SA-coated plates with retention of their responsiveness to stimuli and a differential response to different glycans. This suggests immune cell phenotype can be modulated by glycans in the biomaterial-adsorbed protein layer that is controllable by defined glycan presentation.

References: 1. Shankar SP et al. JBMRA, 2009, DOI # 10.1002/jbm.a.32457
2. Yoshida M, Babensee JE, JBMRA, 2004, 71A: 45-54.

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