Effect of Surface Chemistry on Dendritic Cell Responses and Profile of Carbohydrates Associated with Adsorbed Proteins

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Introduction: For a biomaterial-centered approach for controlling immune responses to combination products, the mechanisms by which antigen-presenting dendritic cells (DCs) recognize and respond to biomaterials needs to be elucidated. We are examining the pattern recognition receptors and the biomaterial-associated molecular patterns which may be involved in mediating DC recognition and response to biomaterials. Profiles of glycosylations associated with proteins adsorbed to SAM surfaces of alkanethiols of different terminal groups were probed for using Enzyme-linked Lectin Assays (ELLA) and correlated with the resultant DC phenotype.

Methods: Preparation and Characterization of SAMs: SAMs were prepared on Au/Ti-coated glass or polystyrene surfaces, by assembly of 1mM solutions of alkanethiols overnight (1). SAM surfaces were characterized by X-ray photon spectroscopy (XPS), contact angle determination and endotoxin content.

Characterization of carbohydrate profiles on SAMs: ELLA: An ELLA was performed using biotinylated lectin probes specific for different carbohydrates, following pre-incubation of SAMs with human plasma or human serum (2). Surfaces were blocked using bovine serum albumin (BSA) in PBS (1hr, 37°C), probed for using biotin-labeled lectin (*Narcissus pseudonarcissus;* NPA, *Ulex europaeus I ;*UEA-1, *Sambacus nigra*; SNA-1,*Ulex europaeus II;* UEA-2, *Peanut agglutinin ;*PEA, or *Hippeastrun hybrid;* HHA: EY labs, San Mateo,CA) (2hrs, 37°C), incubated with avidin labeled with alkaline phosphatase (1hr, 37°C) and detected with a colorimetric enzymatic substrate (1hr, 37°C) with absorbance read at 405 nm.

Enzyme-Linked Immunosorbent Assay (ELISA): An ELISA was performed to determine the amounts of total human IgG and human serum albumin (HSA) associated with SAM surfaces (3). For IgG measurement, surfaces were pre-incubated with human serum or plasma (1hr, 37°C), blocked (1hr, 37°C), incubated with 1:1000 dilution of alkaline phosphatase labeled polyclonal goat anti-human IgG, gamma chain specific primary antibody (Sigma) (2hrs, 37°C), and incubated with colorimetric substrate (1hr, 37°C). For HSA determination, after pre-incubation, SAM surfaces were blocked and treated with 1:1000 dilution of monoclonal mouse anti-HSA (Sigma) (2hrs, 37°C), incubated with 1:1000 dilution of alkaline phosphatase labeled goat anti-mouse IgG (Sigma) (2hrs, 37°C), and treated with substrate.

Dendritic cell culture: Dendritic cells derived from peripheral human blood mononuclear cells (4) were cultured on SAM surfaces. Morphology was determined using cytospin images, activation markers were assessed using flow cytometry and an allostimulatory Mixed Lymphocyte Reaction (MLR) was performed.

Results/Discussion: Differential carbohydrate profiles were detected on different SAM surfaces. NH_2 SAM surfaces had highest amounts of mannose, N-acetylglucosamine and sialylated groups, while CH_3 surfaces had lowest sialylated groups present. Normalization against IgG and HSA amounts, while not reaching significance, verified these results along with images of fluorescent-labeled carbohydrate on SAMs. DCs cultured on NH_2 or COOH SAM surfaces appeared more mature than those cultured on CH_3 SAM surfaces based both on morphology and expression of CD40, CD80 (NH_2 > immature DC (iDC)), expression of HLA-DR (CH_3,OH < iDC) and (COOH, NH_2 > CH_3). The MLR showed that DCs cultured on COOH SAM surfaces were more allostimulatory than those cultured on CH_3 surfaces (Figure 1).

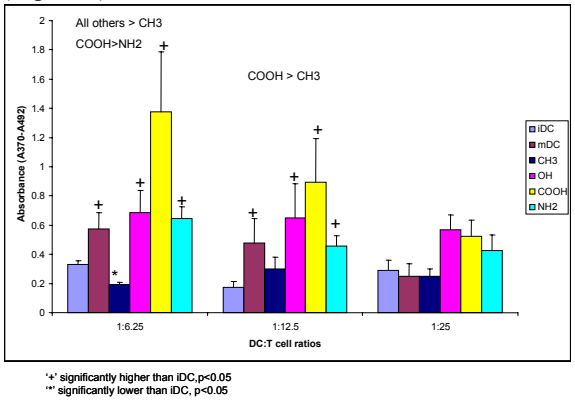

Figure 1: Dendritic cells cultured with SAM surfaces exhibit differential allostimulatory profiles in an MLR. Representative data of three experiments, mean ± S.D.; p<0.05.

Conclusions: NH_2 and COOH SAM surfaces triggered higher DC activation than CH_3 SAM surfaces. While NH_2 SAM surfaces in general had a higher detectable protein glycosylations compared with CH_3 surfaces, COOH surfaces while activating DCs, did not have similar carbohydrate profiles as NH_2 SAM surfaces. This suggests that different mechanisms may be involved in promoting DC activation, or that other factors such as differential profiles of adsorbed proteins and therefore differential spatial distribution of presented carbohydrates that involve the engagement of different receptor groups, ultimately leading to varying cell responses.

Acknowledgements: This research is supported by the NSF CAREER grant (BES-0239152). Timothy Petrie acknowledged for supply of Au/Ti-coated substrates.

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