Effects of Surface Chemistry and Glycosylation Profiles of Adsorbed Proteins on Dendritic Cell Maturation

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Introduction: Dendritic cells (DCs) are key antigen presenting cells that recognize Pathogen Associated Molecular Patterns using Pattern Recognition Receptors (PRRs) (1-3). Our hypothesis is that DCs respond to biomaterials by interacting with 'biomaterial associated molecular patterns'. Understanding these interactions will allow for a biomaterial-centered approach for controlling immune responses to combination products.

Methods: Preparation and Characterization of SAMs: Glass or polystyrene base surfaces were coated with Au/Ti and alkanethiols presenting different endgroups were allowed to self-assemble overnight (RT) (4). SAMs were characterized by XPS, contact angle measurements and assessment of endotoxin levels.

Characterization of carbohydrate profiles on SAMs: A surface Enzyme Linked Lectin Assay (ELLA) was developed based on (5) to detect carbohydrate ligands of DC PRRs presented on adsorbed proteins. SAM endgroups pre-incubated with human serum or plasma were incubated with lectin probes with known carbohydrate specificities (EY labs, CA) (2hrs, 37°C), detected using a biotin-avidin alkaline phosphatase system (1hr, 37°C). The ELLA asborbances were normalized to adsorbed protein amounts from ELISAs for human IgG or Human Serum Albumin (HSA).

DC culture and characterization of DC phenotype: DCs derived from peripheral human blood mononuclear cells were cultured (6) on SAM surfaces. The DC phenotype was assessed for morphology in cytospins, activation marker expression by flow cytometry (FC) and allostimulatory capacity using a Mixed Lymphocyte Reaction. Secretion of pro- or anti-inflammatory cytokines were measured using ELISAs and apoptosis was determined by annexinV/propidium iodide staining, ELISAs for caspase proteins and Terminal transferase dUTP nick end labeling (TUNEL) assay. Levels of shed Tumor Necrosis Factor Receptor Inhibitor (sTNF RI) and shed Cytotoxic T Lymphocyte Associated Antigen receptor-4 (CTLA-4) were measured using ELISAs. Expression of bound CTLA-4 was measured via FC.

Partial de-glycosylation of FBS: To examine the role of carbohydrates in DC maturation, FBS was partially de-glycosylated of carbohydrate ligands of DC C-type Lectin Receptors (CLRs), a family of PRRs. Briefly, N- and O-linked glycosylations were cleaved (Enzymatic CarboRelease Kit ™, QA Bio) and these cleaved carbohydrate moieties were removed by sequential flows through lectin gel columns (EY labs) using lectins as in ELLAs. Processed FBS was characterized for total protein, total glycoprotein contents and specific carbohydrate presence (InstaChek™ Carbohydrate Kit, EY labs). Finally, partially deglycolsated FBS was added to DC media for culture on SAMs, with DC media containing unprocessed FBS as a culture control.

Results/Discussion: Differential profiles of carbohydrate ligands were observed on different SAMs. The NH_2 SAM was associated with highest mannose and complex mannose, CH_3 SAM with lowest sialylated groups, COOH SAM with higher α-galactose than CH_3 or OH SAMs and higher ß-galactose than NH_2 SAM. The DCs cultured on CH_3 SAM were least mature based on morphology, activation marker expression and allostimulatory capacity, but surprisingly, were associated with highest pro-inflammatory cytokine release. To investigate the mechanism leading to this discrepancy, the levels of immunosuppressants and stimulators of anti-inflammatory alternatively activated DCs such as TGF- ß and IL-10 or PGE_2 respectively, that may be present in high levels on CH_3 SAM and counteract effects of pro-inflammatory cytokines, were measured. However, neither of these mechanisms was involved in DC immunosuppression. Interestingly, higher anti-inflammatory caspase-8-mediated apoptosis was measured on CH_3 SAM compared to other endgroups. This may explain lower DC maturation on these surfaces, while high levels of TNF- α were present. Although TNF- α is a strong DC maturation stimulus, it is also a potent inducer of apoptosis. Finally, levels of sTNF RI that negatively regulates TNF- α–mediated apoptosis by competitively binding sTNF- α was measured. As we did not observe lower sTNF RI on CH_3 SAM thereby accounting for higher measured sTNF- α, this mechanism of apoptosis induction was not implicated. Apoptosis may have been induced by classical ligation of HLA-DQ, a robust inducer of DC apoptosis, which had highest expression on DCs cultured on CH_3 SAM compared to all other SAMs. Furthermore, a CTLA-4-mediated mechanism of DC immunosuppression may also be involved on CH_3 SAM. Preliminary results suggest that culture with de-glycosylated FBS slightly increased background iDC maturation, but strikingly lowered LPS maturation effects and overwhelmed any biomaterial effects on maturation marker expression.

Conclusions: Lower DC maturation on CH_3 SAM may be due to induction of apoptosis, as phagocytosis of apoptotic DCs has strong tolerizing effects on DCs.

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