ENGINEERING BIOMIMETIC SUBSTRATES FOR IMMUNOTHERAPIES

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Background: Manipulating the body's immune response to boost or repair natural healing mechanisms is a powerful approach that is being explored in current clinical treatments using dendritic cell (DC) immunotherapies. However, limitations in current DC-based therapies have prevented broad success. In order to address these limitations, this project investigates the ability of integrin receptor binding to direct DC maturation. Integrins represent the family of cell-surface receptor primarily responsible for cell adhesion to extracellular matrix adhesion proteins and also regulate numerous cell-cell interactions. [1] This is a neglected area of research, as most reports describe DC integrins only as markers characterizing different tissue and maturation specific DC subsets. However, it has been established that these different DC subsets, defined in part by expression of certain integrins, give rise to diverse immune responses. There is therefore enormous potential to utilize the functional role of integrins in DC activation. Our goal is to engineer integrin-targeting cell adhesive substrates targeting DC integrins, representing the first systematic analysis of the effects of integrin adhesion on DC activation. This project addresses limitations in current DC-based therapies and it is expected that results will be applicable to immunotherapies currently used clinically, in particular, those aimed at the management of cancer, transplantation and autoimmunity.

Methods: Dendritic cells were matured from extracted bone marrow stem cells of wild type C57BL6 mouse and incubated with IL-4 and GMCSF for 5 days. Affinity chromatography was employed to separate undifferentiated cells from DCs. DCs were then seeded onto tissue culture-treated polystyrene coated with either fibronectin, collagen, or 10 % serum with LPS (lipopolysaccharide). ELISA was performed on the supernatant for quantification of IL-10. Flow cytometry was carried out to quantify surface presentation of CD80 and MHC-II as a marker of DC activation.

Results & Discussion: Quantification of IL-10 production a marker of DC activation revealed that DCs cultured on fibronectin produced levels approximately two-fold over DCs cultured on type-I collagen, and an equivalent amount compared to LPS (**Figure 1**). DC on phagocytosing the antigen digest it into peptides which are displayed on its surface bound to MHC class II molecules. Also, on activation there is an upregulation of co-stimulatory molecule CD80.). Flow cytometry analysis also demonstrated surface expression of MHC class II (involved in antigen presentation) and CD80 (co-stimulatory) proteins. The difference between the control and the matured DCs is evident from flow cytometry results shown in **Figure 2**. **Figure 2a and 2b** show dendritic cells cultured on fibronectin and serum added LPS double positive for CD80 and MHC II, the surface expression of which indicates mature DCs. Where as immature DCs are double-negative for both these cell surface molecules.

This preliminary data suggests that signals from extracellular matrix proteins provide activation cues, which is corroborated by other reports investigating DC adhesion to extracellular matrix proteins[2] and polymeric surfaces.[3] The next step of this project incorporates integrin-targeting adhesive peptides such as RGD (targeting $\alpha_V\beta_1$, $\alpha_V\beta_3$, $\alpha_E\beta_7$ integrins), GFOGER (targeting $\alpha_2\beta_1$, $\alpha_1\beta_1$ integrins) and HYD1 (targeting $\alpha_6\beta_1$, $\alpha_3\beta_1$ integrins) to differentially activate DCs. Peptides will be presented using self assembled monolayers of alkanethiols on gold surfaces which enable presentation of controlled density of integrin-targeting peptide against a cell adhesion-resistant background. This will enable us to analyze the effect of integrin binding specificity on DC activation and maturation.

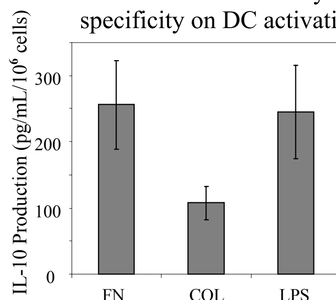

Figure 1. Analysis of cytokine production by bone marrow-derived cells cultured on various adhesive substrates, fibronectin (FN) and collagen (COL) and stimulated with LPS (positive control), ANOVA $p<0.04$.

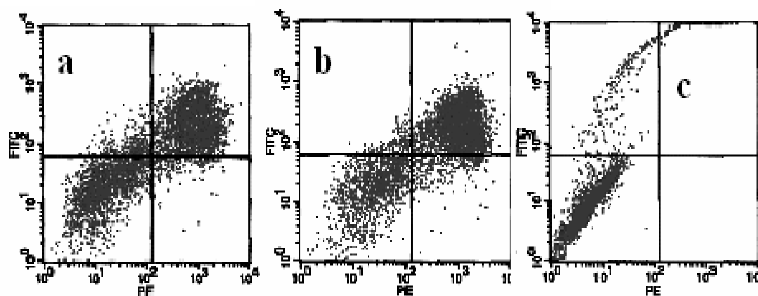

Figure 2. Representative figures of the study of surface markers of the matured dendritic cell using FITC-CD80 and PE-MHC II . Figure (a) shows the presence of CD80 and MHC-II molecules on the surface of the DCs, cultured in fibronectin. Figure (b)serum+LPS shows similar response to fibronectin (c) immature DCs do not express these surface markers.

Reference:

[1] Hynes et al *J Clin Invest.* (1999); [2] Mahnke *J Leukoc Biol.* (1996); [3] Babensee *J Biomed Mater Res A*. (2005)