The Role of Integrins in the Recognition of Biomaterials by Dendritic Cells

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Statement of Purpose: Dendritic cells (DCs) are the gatekeepers to the initiation of immune responses. The central role of DCs in balancing immunity and tolerance renders the control of their phenotype particularly important in situations where immune responses are harnessed in combination products, such as vaccine delivery systems or tissue engineering strategies. The mechanism by which DCs interact with biomaterials remains to be elucidated. Herein, we investigated the role of β1 and β2 integrins in mediating DC interactions (adhesion and maturation) in response to biomaterials using monoclonal antibody function blocking treatments. Integrins mediate leukocyte interactions with endothelial cells in the inflammatory cascade as well as adhesion to biomaterials but their contribution to DC interactions with biomaterials is unknown.

Methods: PLGA (75:25) (Lactel Absorbable Polymers) films were prepared by solvent casting and immediately prior to treatment of DCs, exposed to UV for 30 min on each side. Endotoxin content of films was tested (QCL-1000 LAL Assay, Lonza) and measured below 0.1 EU/ml. Human peripheral blood mononuclear cells were differentiated into immature DCs (iDCs) in media containing GM-CSF and IL-4 cytokines for 5 days. Loosely-adherent DCs were collected and pre-treated with 10μg/ml, 20μg/ml or 40μg/ml of either purified mouse IgG1, κ isotype or mAb toward CD18/β_2 (TS1/18) (both from Biolegend) (1hr, 37C). DCs were then cultured on TCPS or treated with PLGA films. Cell adhesion and spreading were observed at 3hr and 24hr using phase contrast microscopy. At 24hr, loosely/non-adherent cells were collected and counted using Multisizer 3. Adherent cells were collected using cell dissociation buffer (Sigma) and similarly counted. Non-adherent and adherent cells were pooled and stained for CD18/β_2 to assess effective blocking as well as CD86 to assess DC maturation. In a separate experiment, expression of integrins (CD29/β_1 and CD18/β_2) as well as DC marker DC-SIGN and CD86 was determined using immunofluorescence of TCPS or PLGA-adherent DCs.

Results: DCs treated with PLGA films adhered and spread with pronounced dendrite processes, at 3 and 24hrs. This was in contrast to DCs on TCPS which showed fewer adherent DCs with a more rounded morphology. Pre-treatment of DCs with anti-CD18/β_2 resulted in DCs of a rounded morphology upon treatment with PLGA and no dendritic processes were observed (Fig. 1). Pre-treatment of DCs with anti-CD18/β_2 significantly reduced adherence of DCs to PLGA or TCPS as for both materials twice as many loosely/non-adherent DCs were recovered as compared to the isotype control pre-treatment (Fig. 2). Effective and specific blocking of CD18/β2 on DCs by anti-CD18/β_2 pre-treatment (not isotype pre-treatments) was confirmed by flow cytometric analysis using fluorescently-labeled antibodies toward CD18/β2. Interestingly, expression of DC maturation marker CD86 was found to decrease significantly on DCs pre-treated with anti-CD18/β2 (at all concentrations) and cultured on TCPS (in comparison to pre-treatment with isotype controls). A lowering of CD86 expression was also observed for DCs pre-treated with anti-CD18/β2 (at 40μg/ml only) before treatment with PLGA films but this result was not statistically significant.

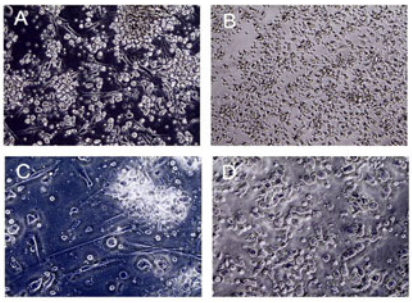

Figure 1: Micrographs of DCs on TCPS+ 20ug/ml Isotype (A), TCPS + 20ug/ml anti-CD18 (B), PLGA+20ug/ml Isotype (C), PLGA+20ug/ml anti-CD18 (D) at 24hr (10X mag.)

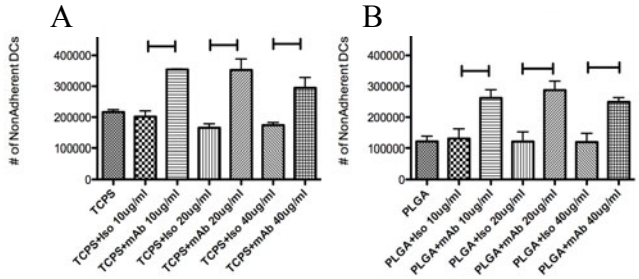

Figure 2: Number of non-adherent DCs harvested from TCPS (A) or PLGA (B) surfaces following isotype (Iso) or anti-CD18 (mAb) pre-treatment (n=3). Brackets indicate statistical significance between mAb and isotype treatment, $p<0.05$.

Conclusions: Adherence of DCs to biomaterials is CD18/β_2-dependent. Furthermore, adhesion of DCs to a substrate is necessary, at least in part, for their activation by a biomaterial. Blocking CD18/β2-dependent adhesion of DCs to a substrate does not affect the maturation induced by soluble activators of DCs (e.g. lipopoly-saccharide). A direct link between DC adhesion to substrate and maturation has not previously been documented. Future work involves assessing the contribution of β1 integrins to DC interactions with biomaterials and the α subunits for β1 and β2 integrins..

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