**Systems Biology Analysis of Dendritic Cell Responses to Biomaterials**

Fesenkova V., Kemp M.L., Babensee J.E.
Wallace H Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, Georgia Institute of Technology, Atlanta, GA 30332, USA.

**Introduction:** The central role of dendritic cells (DCs) as gatekeepers to the initiation of immune responses and maintenance of tolerance renders the control of their phenotype particularly important in situations where immune responses can be harnessed in combination products, such as vaccine delivery systems or tissue engineering strategies. We have demonstrated biomaterial-based control of DC phenotype, depending on the biomaterial used to treat DCs[1], including modulation of immune responses to co-delivered antigen *in vivo*[2]. Receptor-based mechanisms by which DCs recognize and respond to biomaterials are largely unknown, however commonalities between the cytokine, integrin, and pattern recognition receptor-induced cellular responses and biomaterial-induced DC maturation suggest that DCs engage a *combination* of these external sensing receptor families to initiate phenotypic responses to biomaterials. In this study we established an integrative model of signaling and cytokines profiles for known receptor-driven DC phenotypic outcomes and developed a computational model for DC signaling with prediction of DCs response to biomaterials. The overall goal is to use this model to elucidate the receptor families DCs use biomaterial-induced responses.

**Methods:** Immature DCs (iDCs) were derived from human peripheral blood mononuclear cells (6 healthy donors) during a 5 day culture in the presence of inducing cytokines (GM-CSF, IL-4). iDCs were treated (time points as below) with ligands for key external sensing receptors, LPS, MIP-1α, TNF-α, immobilized-IgG (iIgG), Fibronectin (FN), Zymozan or with biomaterial films known to induce DCs to opposite phenotypic outcomes (PLGA or Agarose), in a 96-well plate high throughput format (HTP)[3]. Controls also included untreated iDCs and IL-10/IFN-α treated tolerogenic DCs (tDCs). Dynamics of signaling phosphoproteins were analyzed after 10min, 2 and 12 hr of treatment using a MILLIPLEX $_{MAP}$ Signaling MAPmate Kit for AKT, ERK1/2, p38, JNK, NF-kBp65, IkBa, Syk. Following 1, 12 and 24 hrs of treatment, DC expression of CD86, ILT3, DC-SIGN was determined using HTP immunofluorescence and cytokine production (IL-10, MIP-1α, IL-1RA, TNF-α, IL-1β, IL-15, IL-12p70) was analyzed by multiplex immunoassay. Multivariate analysis was performed using the software SIMCA P+ (Umetrics, Malmö, Sweden) as previously described[4].

**Results:** Ligands for DC treatment were classified as pro-inflammatory (LPS, TNF-α, iIgG, Zymozan) causing DC maturation and anti- inflammatory (tDC, MIP-1α, FN, anti-gC1qR) inducing more tolerogenic DCs. This was clearly demonstrated with application of principal component analysis (PCA) separately discriminating pro-inflammatory from tolerogenic treatments. DC CD86 expression was associated with pro-inflammatory treatments while tolerogenic treatments were associated with ILT3 expression and these markers were anti-correlated. This PCA approach was used to map the biomaterial-induced DC observations to those of ligand treatment. The model suggested that DCs treated with PLGA closely covaries with DCs treated with either TNF-α or iIgG while DCs treated with agarose covaries with DCs treated with FN (Figure 1). To identify the phenotypic outcomes that are most predictive of DC responses to biomaterials, Partial Least Square Regression was used to train a model of known ligand treatments and regress against various cytokine and surface marker outcomes. With each biomaterial as an independent set, DC response to PLGA was best predicted through the phosphorylation patterns associated with TNF-α cytokine production ($R^2Y$=0.80); DC response to agarose was best predicted by a model of IL-1RA production ($R^2Y$ =0.86).

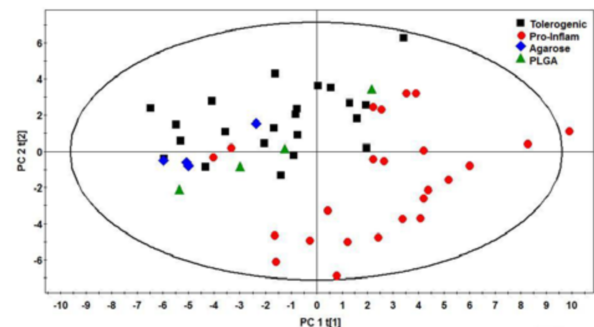


**Figure 1:** PCA model illustrating extracted relationships between Agarose-FN and PLGA-TNFα or iIgG. Model developed with following variables: CD86/DC-SIGN, ILT3/DCSIGN, phosphoproteins, IL-10, TNF-α. Observations were grouped as tolerogenic (black): iDC, tDC, MIP-1α, FN; Pro-inflammatory (red): LPS, iIgG, Zymozan, TNF-α; with biomaterials indicated as agarose (blue) and PLGA (green).

**Conclusions:** The strong correlation between the observations associated with PLGA-treated DC with that of iIgG or TNF-α, suggest an interesting hypothesis towards explaining the maturation effect of this biomaterial. Presumably DCs are influenced by interactions with IgG adsorbed to this biomaterial, either directly through FcR or via complement activation effects, resulting in autocrine maturation factor, TNF-α, release. Agarose treatment of DCs is correlated with FN treatment of DCs, a protein previously shown to not cause DC maturation[5]. The understanding the interactions of DCs with biomaterials at the receptor level will inform which receptors should be engaged by ligands engineered into biomaterials to induce specific DC responses. In this way, the phenotype of DCs can be specifically engineered to modulate the resultant immune response to an associated antigen for either immunity or tolerance.

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**References:** 1. Babensee JE, Paranjpe A, J Biomed Mater Res, 2005, 74A: 503. 2. Norton L, Park J, Babensee JE, J Control Rel, 2010, 146:341. 3. Kou PM, Babensee JE, Biomaterials 2012, 3:1699. 4. Rivet CA, Hill AS, Lu H, Kemp ML, Mol Cell Proteom, 2011, 10.1074/mcp.M110.003921–1. 5. Acharya AP., et al. Biomaterials, 2008, 29:4736.


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