Differential Macrophage Activation Due to Biomaterial Topography Requires the B2-Integrin Signaling Pathway

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Introduction: The host response to a biomaterial can be affected by surface topography. For example, tissue surrounding expanded polytetrafluoroethylene (ePTFE) implants has greater vascularity and a less organized, less thick capsule compared to smaller pore ePTFE.[1,2] *In vitro* macrophage activation has also been shown on ePTFE materials.[3] This differential biological response motivated our mechanistic study of *in vitro* macrophage activation on biomaterials of differing PTFE membrane topography. Cytokine production, cell shape, proinflammatory gene expression and integrin signaling were examined.

Methods: Primary human monocytes were isolated and plated on materials that differed only by topography: biaxially-expanded PTFE filters (ePTFE, 4.4um intranodal distance; Millipore, Bedford, MA) and nonporous PTFE (Berghof/America, Coral Springs, FL). Cell surface area was measured using scanning electron microscopy. To block actin polymerization, monocytes were treated with varying concentrations of cytochalasin D prior to seeding and during culture. To examine the role of integrin receptors, monocytes were treated with anti-integrin blocking antibodies (anti-β1, anti-β2, or anti-αvβ3) or an isotype-matched control antibody (IgG1) prior to plating. After seeding for 24 hrs, adherent cell number was assessed using the lactate dehydrogenase (LDH) assay, and interleukin 1-β (IL-1β) was measured by ELISA. Results are expressed as a percentage of the values for untreated cells on ePTFE (mean +/- SEM for three or more independent donors). Alternatively, total RNA was isolated at 24 hrs after seeding for use in cDNA microarray experiments; results are for two donors.

Results / Discussion: *In vitro* activation of monocytes/macrophages on ePTFE and PTFE was examined. Higher amounts of IL-1β were produced by monocytes cultured on ePTFE compared to those on PTFE, demonstrating a strong topographical response (Figures 1 and 2, untreated samples).

In addition, cells were half as spread on ePTFE as they were on nonporous PTFE, indicating a role for the actin cytoskeleton in the cytokine response. Treatment of primary monocytes with cytochalasin D further demonstrated the role of the actin cytoskeleton. Increasing amounts of cytochalasin D reduced cell adhesion on both materials (data not shown). IL-1β production by monocytes on ePTFE also decreased in a cytochalasin D dose-dependent manner, with IL-1B production reduced at 80 or 125 nM ($p<0.05$) of cytochalasin D (Figure 1).

Differential gene transcription between cells on the two materials was measured using microarrays and agreed with the cytokine ELISA data. Monocytes/macrophages on ePTFE had greater than two-fold higher expression of proinflammatory cytokines and chemokines, including IL-1β, tumor necrosis factor (TNF), monocyte chemotactic protein 1 (MCP1), and macrophage inflammatory protein 1–β (MIP1-β).

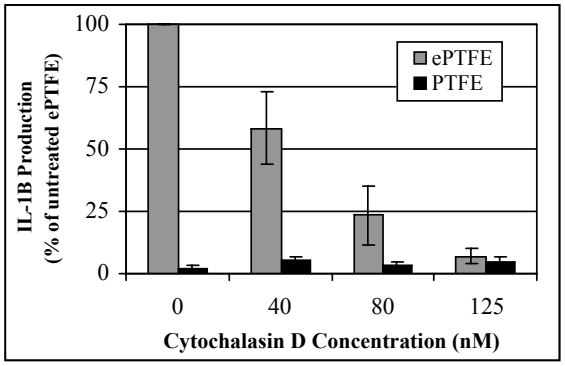

Figure 1. Blocking actin polymerization with cytochalasin D decreases production of IL-1β by primary human monocytes plated on ePTFE.

Transcription of these genes can be induced by β2 integrin receptors.

The transcriptional upregulation of proinflammatory genes and the actin cytoskeleton involvement implicated a direct role for integrin receptors. Treatment with anti-β2 antibodies reduced cell adhesion to 70% on ePTFE and 54% on PTFE ($p<0.05$) while anti-β1 and anti-αvβ3 antibodies had no effect (data not shown). Anti-β2 blocking antibodies also reduced IL-1β production on ePTFE materials to a basal level of 10% +/- 6.2 ($p=0.0001$) of the production in untreated cell populations plated on ePTFE, while anti-β1, anti- αvβ3, and a control antibody did not (Figure 2). This result demonstrates that functional β2 integrin receptors are necessary for macrophage activation on ePTFE.

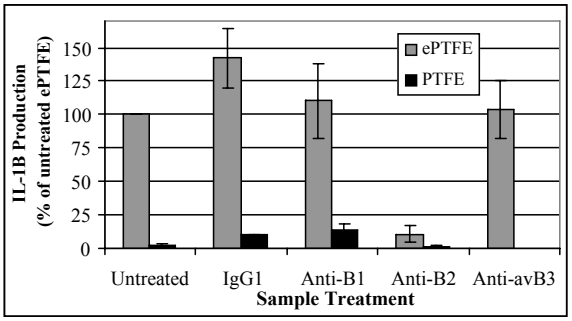

Figure 2. Antibody blocking of β2 integrins reduces IL-1β production by monocytes on ePTFE.

Conclusions: The actin cytoskeleton and β2 integrin receptors are mechanistically tied to the differential activation and proinflammatory cytokine production of human monocytes in response to PTFE membrane topography.

References:

1. Brauker JH et al. J Biomed Mater Res 1995;29:1517.
2. Sharkawy AA et al. J Biomed Mater Res 1998;40:586.
3. Miller KM et al. J Biomed Mater Res 1988;22:713.