Macrophage Phenotypic Stability During Extended Culture

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Statement of Purpose: Implanted biomaterials cause chronic and acute host inflammatory response followed by atypical fibrosis at the wound site. This process, known as the foreign body response (FBR), is often responsible for device failure and its importance increases as the use of medical implants continues to rise. Host reactions that produce the FBR are poorly understood, but are correlated with macrophage attachment to the biomaterial surface and subsequent release of inflammatory cytokines.[1] The work described here is aimed at expanding our knowledge of macrophage response to surfaces. We report changes in morphology and cytokine release over time for adherent macrophages with minimal changes in cell surface markers. It has been opined that changes in morphology and signaling indicate the presence of a phenotypic switch.[2] However, our data indicate that these cells largely maintain their cell surface molecules, an important cellular phenotype component.

Methods: Primary-derived bone marrow macrophages (BMMO) harvested from C57/BL-6 mice (Jackson Labs) and murine monocyte/macrophage cell lines (IC-21, J774A.1 (J7), and RAW 264.7 (RAW)) (ATCC, Manassas, VA) were cultured under standard serum conditions. Surfaces (tissue culture polystyrene, PLLA, and Teflon-AF) were purchased or prepared as previously described.[3-5] Cells were cultured on all surfaces continuously for 21 days. Media was changed daily, and analyzed for expression of cytokines involved in inflammation and the FBR (GM-CSF, IFN-γ, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, MCP-1, MIP-1β, and TNF). Cells were imaged daily to observe morphology. Prior to seeding and at day 21 cells were probed for surface markers of macrophage phenotype, adhesion, and activation (F4/80, CD14, CD11b, CD11c, CD18, CD54, Fc Receptor, Macrophage Mannose Receptor, CD40, and TLR-4) with flow cytometry.[3-5]

Results: Microscopic evaluation of BMMO cells showed surface-dependent changes in morphology enhanced over time. In contrast, murine cell lines developed a rounded or cobblestone morphology on all surfaces. Multiple cytokines involved in inflammation and the FBR were probed over the duration of the extended culture. Of the cytokines assayed, only 3 were detected in BMMO cells (IL-6, MCP-1, and RANTES) while cell lines had slightly more signaling molecules (TNF and MIP-1β) detected. Normalized to cell numbers, these cytokines showed very similar patterns of expression: an initial burst of expression followed by reduction down to basal levels (Figure 1) over time. Intensity of the initial burst of expression among cell lines is correlated to their observed differentiation state, with highly differentiated cells (BMMO, IC-21) expressing much more cytokine than less differentiated (more monocytic) cells (J7, RAW) (Figure 1). Considering expression of surface markers for macrophage phenotype, adhesion, and activation, very few changes are observed following 21 days of continuous culture (Figure 2). The few changes observed, particularly the increase of MMR on all surfaces for all cell types, point to further differentiation of the cells and alternative activation, this type of activation has been previously associated with the FBR.[1,2]

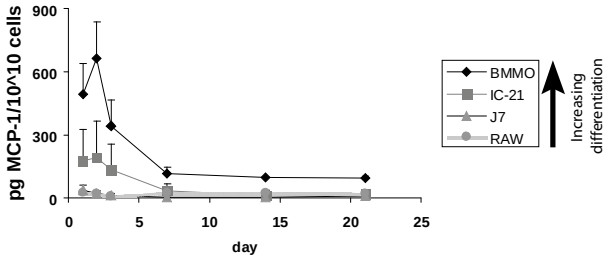

Figure 1: MCP-1 expression per 10^10 cells over time.

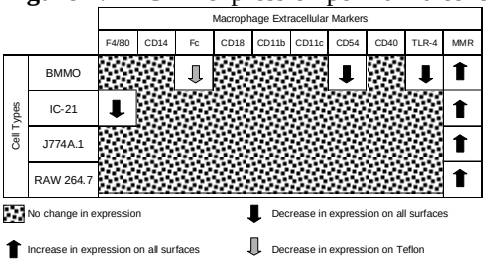

Figure 2: Changes in the expression of molecular markers following extended culture of macrophages

Conclusions: Changes in morphology and cytokine expression following extended culture on materials point to a change in the molecular phenotype of these cells, yet macrophage phenotype remains relatively stable. This observation leads us to hypothesize that either macrophages have a pre-programmed response following adherence to surfaces which does not cause changes in phenotype at the molecular level, or that they require outside signaling from other cells to change their phenotype *in vivo*. Data also suggest that each cell line activates differently on surfaces and over time.

References: 1. Anderson, J.M., Annu. Rev. Mater. Res., 31, 81, (2001); 2. Anderson JM, Jones JA. *Biomaterials*. in press (2007); 3. Godek ML, Sampson JS, Duchsherer NL, McElwee Q, Grainger DW. *J Biomat Sci Polym Ed*, 17, 1141, (2006); 4. Godek ML, Michel R, Chamberlain LM, Castner DC, Grainger DW. in press (2008). *J Biomed Mater Res A*; 5. Chamberlain LM, Godek ML, Gonzalez-Juarrero M, Grainger DW. in press. *J Biomed Mater Res A* (2008).