Modulation of Human Monocyte Phenotypic Polarization by Biomaterials and Xenoproteins

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Statement of Purpose: Monocyte-derived macrophages are critical in the host foreign body response to biomaterials and have been studied extensively in various culture conditions *in vitro*. Since monocyte maturation into macrophages is highly plastic and may vary considerably depending on the surface, isolation procedures, and *in vitro* culture conditions, variations in protein adsorption and serum type may greatly impact monocyte behavior in a surface-dependent manner. The polarization of macrophages has been divided into two categories: classically activated macrophages (M1) and the more diverse subsection of alternatively activated macrophages (M2). M1 cells tend to be more inflammatory while M2 cells are directed towards anti-inflammatory activities. In this study, human blood-derived monocytes were cultured in fetal bovine serum (FBS)- or autologous human serum (AHS)-supplemented medium on PEG hydrogels, polydimethylsiloxane (PDMS), and tissue culture polystyrene (TCPS) with or without the addition of lipopolysaccaride (LPS) to drive M1 phenotype. Phenotypic markers of the monocyte maturation state were then quantified: broadly acting inflammatory cytokines IL-1β, IL-1α, IL-12(p40), IL-12(p70), IFN-γ, and TNF-α; the anti-inflammatory cytokine IL-10; growth factors GM-CSF and TGF-α; and chemokines IP-10, GRO-2, and MCP-1. To further assess the polarization state of the adherent cells, surface CD86 and CD163 markers were quantified. Additionally, the composition of the adsorbed protein layer derived from human serum on both PEG and TCPS surfaces was analyzed and the role of critical proteins in monocyte adhesion was investigated.

Methods: ***Material construction:*** 10 wt% PEG hydrogels were constructed using 575 and 3400 Da PEG diacrylate and 0.1 wt% UV photoinitiator (Irgacure 2959®). PEG hydrogels and medical grate PDMS discs were placed into TCPS well plates with TCPS serving as the third surface. ***Monocyte isolation and culture conditions:*** Human peripheral blood monocytes were isolated from citrated whole blood of a healthy adult volunteer using a density-gradient, non-adhesion method. Cells were seeded on surfaces in RPMI+10% AHS or FBS. Light microscope images were captured for each surface at each time point to quantify adherent cell density. ***Maturation state analysis:*** At each time point, supernatant was collected and assayed for the proteins of interest using a microsphere-based multiplex assay (Bio-Rad, Millipore). At day 7, the adherent cells were fixed in 1% paraformaledhyde (PFA, Polysciences, Inc.) and stained with labeled antibodies directed against CD86 and CD163 and then imaged via fluorescent confocal microscope. ***Protein adsorption analysis***: PEG hydrogels and TCPS were incubated with fractionated and whole human serum and digested peptides of the adsorbed proteins were analyzed using MALDI TOF/TOF. Further quantification of adsorbed vitronectin, thrombin, fibronectin, and C3 was performed using ELISA. To examine the effect of C3 on monocyte adhesion, the effect of C3-inactivated sera was compared to pooled human serum.

Results/Discussion: Adherent monocyte density was 3- and 10-fold higher on TCPS and PEG surfaces, respectively, when AHS was used versus FBS-supplemented medium, while no difference in adhesion density was observed on PDMS. Significant differential variations in protein release were observed between the serum conditions on these surfaces. In particular, there was a 100-fold higher concentration of GRO-2 for the AHS condition on PDMS. GRO-2, IL-1β and TNF-α concentrations were also significantly different between the AHS and FBS conditions in the presence of PEG hydrogels while no difference was observed for IL-1α, GM-CSF, TGF-α, and IP-10. Adherent monocytes on all surfaces and cultured with either AHS or FBS supplementation stained for CD86 and little to no staining was observed for CD163. The results demonstrate that while most of the cells in all conditions exhibit pro-inflammatory markers, the degree of response varied between surfaces and serum conditions. PEG hydrogels and TCPS were found to have differing composition of surface-bound proteins. PEG hydrogels significantly inhibited the adsorption of vitronectin, thrombin, fibrinogen and complement component C3 compared to TCPS. The inactivation of C3 from human serum significantly decreased the number of cells on PEG and especially on TCPS substrates.

Conclusion: The impact of xenoproteins in FBS on the interaction between material and primary human cells is not well documented and this study raises important considerations for the culture medium when comparing literature on monocyte adhesion and inflammatory responses to biomaterials. The decreased C3 adsorption and adherent monocyte density on PEG hydrogel further demonstrate that complement C3 is a key factor in mediating monocytes adhesion and possible maturation on biomaterials. Our study implicates the critical role of the biomaterial surface in tandem with the culture serum in mediating the adhesion and protein release of human blood-derived monocytes.

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