Cytokine Production from Lymphocyte/Macrophage Interactions with Biomaterial Surfaces

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Statement of Purpose: Monocytes/macrophages play an integral part in inflammation, wound healing, and the foreign body reaction (FBR) at the implant site. Monocytes/macrophages are capable of guiding the tissue response through the production of various signaling molecules. The presence of lymphocytes at the implant site as well as IL-4 and IL-13 lymphokine participation in macrophage fusion implicates a critical role for lymphocytes in the FBR. There have been relatively few studies examining lymphocyte activity and interactions at the tissue/material interface. Recent studies in our lab have shown that lymphocytes modulate macrophage adhesion and fusion on biomaterials. This study aims to investigate the effects of biomaterial surfaces on the cytokine response from lymphocytes and monocyte/lymphocyte interactions in order to gain insight into the role lymphocytes play at the biomaterial surface.

Methods: Polyethylene terephthalate (PET)-based surfaces displaying distinct characteristics were utilized. A coating of BDEDTC provided a hydrophobic surface while photograft copolymerization of PAAm, PAANa, and DMAPAAmMeI onto the BDEDTC provided surface chemistries with hydrophilic, hydrophilic/anionic, and hydrophilic/cationic properties, respectively.

Human peripheral blood monocytes and lymphocytes were isolated simultaneously. The lymphocyte population was cultured on the biomaterial surfaces alone and in direct co-culture with the monocyte population in serum-free medium (Invitrogen, Grand Island, NY) containing 20% autologous serum for periods of 3, 7, and 10 days.

Analysis of adherent cell density and fusion were performed after methanol fixation and May-Grünwald/Giemsa staining. Supernatant analysis was performed utilizing RayBio® antibody arrays (RayBiotech, Inc., Norcross, GA) for the detection of 79 cytokine/chemokines/matrix proteins and Quantikine® ELISA (R&D Systems, Minneapolis, MN) for protein quantification.

Results/Discussion: Analysis of surface-adherent cells in lymphocyte/macrophage co-cultures showed that relative to PET, the hydrophilic/neutral surface, PAAm, decreased adhesion at all time points. Additionally, PAAm and the hydrophilic/anionic surface PAANa promoted macrophage fusion. Hydrophobic (BDEDTC) and hydrophilic/cationic (DMAPAAmMeI) surfaces showed no difference in adhesion or fusion.

Antibody array screening resulted in the selection of IL-1β, IL-6, IL-8, IL-10, MIP-1β, TIMP-1, TIMP-2, and MMP-9, and TGF-β2 for further quantification. Significant cytokines not detected in either lymphocyte culture or co-culture supernatants included: IL-2, IL-3, IL-4, IL-5, IL-13, and IFN-γ. The absence of IL-2 in all cultures and time points was confirmed by ELISA.

Over the time course in the co-cultures, IL-1β and MIP-1β levels generally decreased; IL-6, IL-8, IL-10 levels were maintained; and TGF-β2, TIMP-1, TIMP-2, and MMP-9 levels increased. The results show a trend towards an increased production of inflammatory cytokines, IL-1β, IL-6, and IL-8, on the hydrophilic surface (PAAm). Moreover, the hydrophilic surface, along with the hydrophilic/anionic surface (PAANa), tends to promote a decreased MMP-9/TIMP-1 ratio and increased TIMP-2 levels. Conversely, the hydrophilic/cationic surface (DMAPAAmMeI) promotes an increased secretion of IL-10, an anti-inflammatory cytokine, with an increased MMP-9/TIMP-1 ratio and decreased TIMP-2 levels. Finally, MIP-1β levels were highest on hydrophilic (PAAm) and anionic (PAANa) surfaces corresponding to fusion levels and decreased with time as fusion increased (Figure 1).

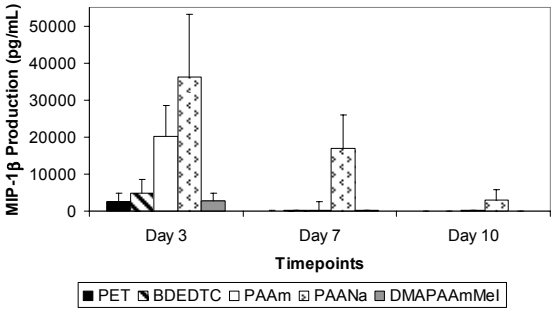

Figure 1. Co-culture MIP-1β production

Conclusions: Lymphocytes do not appear to be immune activated by macrophages or PET-based surfaces *in vitro*, although the detection of signaling molecules in lymphocyte cultures suggests they play a role in the tissue response. Lymphocytes and macrophages in co-cultures demonstrate material surface-dependent production of cytokines, chemokines, and matrix proteins. The hydrophilic surface (PAAm) appears to promote a pro-inflammatory response while decreasing matrix degradation. Conversely, the cationic surface (DMAPAAmMeI) promotes an anti-inflammatory response and an environment of increased ECM breakdown. Finally, the research suggests further investigation into: (1) direct and indirect lymphocyte and macrophage interactions on biomaterials and (2) MIP-1β as a possible fusion promoting factor and a potential target for minimizing the FBR.