Proteomic Analysis and Quantification of Cytokines and Chemokines from Biomaterial Surface-Adherent Macrophages and Foreign Body Giant Cells

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Statement of Purpose: Implantation of biomaterials results in the well-known foreign body reaction consisting of monocytes, macrophages, and foreign body giant cells (FBGCs) at the material/tissue interface. These cells can produce a variety of cytokines and chemokines that modulate inflammatory and wound healing cells in the surrounding milieu ultimately directing responses such as inflammation, the foreign body reaction, wound healing, angiogenesis and fibrous encapsulation. If material surface chemistry can affect the production of the macrophage/FBGC derived cytokines and chemokines, then the subsequent biological responses to biomaterial implants could be directed accordingly. In this research, we continue to address the hypothesis that material surface chemistry modulates the phenotypic expression of these cells via the objectives to proteomically identify and further quantify cytokine/chemokine profiles secreted from macrophages/FBGCs adherent to biomaterials of distinct surface chemistry and to analyze material-dependent cellular activation based upon these profiles.

Methods:

Polyethylene terephthalate (Mylar®, PET) modified materials were coated with BDEDTC and photografted with one of following: acrylamide (PAAm), sodium salt of acrylic acid (PAANa), or methyl iodide salt of N-[3-(dimethylamino) propyl] acrylamide) (DMAPAAmMeI) as described elsewhere[1]. Material surface chemistries were confirmed using advancing water contact angles, acid and base reacting dyes for ionic chemistry, and ATR-FTIR spectroscopy.

Freshly isolated human monocytes were cultured onto biomaterials in media containing 20% autologous serum as described previously[2]. Cultures were continued until 3, 7 and 10 days when supernatants were collected, adherent cells in select cultures were fixed with methanol for adherent cell density analysis, and remaining cultures were continued.

Human antibody arrays were utilized to detect 77 cytokines/chemokines in cell culture supernatants. Select proteins were further quantified using ELISAs. Adherent cell densities were measured in May-Grunwald and Giemsa stained samples. The amount of cytokine or chemokine produced per cell was determined via the normalization of the concentration with the adherent cell density for analysis of cellular activation.

Results/Discussion:

Approximately 24 cytokines/chemokines were present in the cell culture supernatants at day 3. Initially, these profiles contained many of the cytokines and chemokines produced by classically activated macrophages. By day 10, the profiles were more similar to alternatively activated macrophages suggesting that the macrophages underwent a phenotypic switch. ELISA quantification showed that the concentrations of pro-inflammatory cytokines IL-1β and IL-6 and chemokines MIP-1b and IL-8 decreased over time, while the anti-inflammatory cytokine IL-10 continued to be produced between days 3 and 10 (FIGURE 1). Material chemistry did not significantly affect cytokine and chemokine concentrations universally, but unique trends for each cytokine and chemokine did exist.

Analysis of the amount of cytokine/chemokine produced per cell showed that although the hydrophilic/neutral surface (PAAm) inhibited adhesion, cells on the PAAm surface produced 8 to 51 fold greater amounts of various cytokines per cell when compared to adherent cells on the other surfaces.

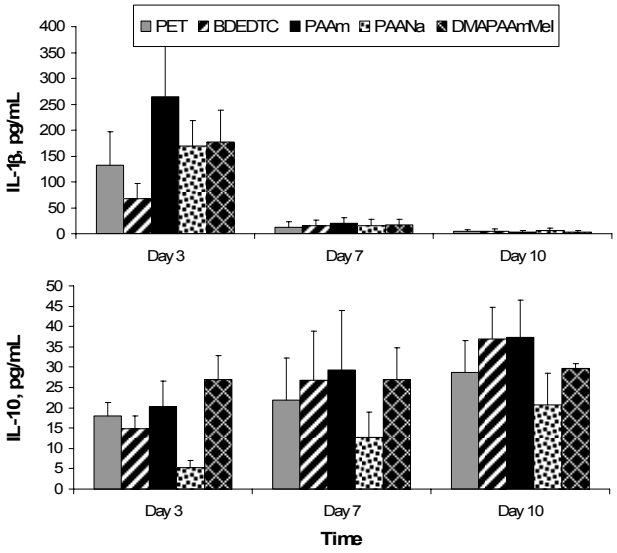

Figure 1. IL-1β and IL-10 Concentrations Over Time

Conclusions:

This study clearly presents evidence that material surface chemistry can differentially affect cytokine and chemokine profiles derived from macrophages and FBGCs adherent to biomaterial surfaces. In addition, it demonstrates that hydrophilic/neutral surfaces further activate these cells to produce greater amounts of cytokines/chemokines on a cell to cell basis. Finally, this research prompts further investigation into a potential phenotypic switch from pro- to anti- inflammatory that occurs in biomaterial adherent macrophages.

References: [1]Nakayama Y. JBMR. 2000;53:584-591
[2]Jones JA. J Biomater Sci Polymer Edn. 2004;15:567-584