Quantification of Exudate T Lymphocyte Subpopulations to Biomaterial Implants by Flow Cytometry

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Statement of Purpose: Investigation of the complex host response to biomaterials is necessary in order to engineer materials with desired biocompatibility properties. The rat cage implant system is a well established *in vivo* model to investigate inflammatory cell infiltration to the biomaterial implant site. In this study, flow cytometry was used to quantitate exudative cells surrounding three clinically relevant materials. T lymphocyte subpopulations were analyzed because the role of T lymphocytes in the inflammatory response to biomaterials has been minimally studied. *In vitro*, lymphocytes augment monocyte adhesion and fusion on material surfaces. Biomaterial surface chemistry has been found to influence monocyte/lymphocyte intereactions *in vitro*. The goal of this study was to identify material dependent variations in exudates T cell populations *in vivo* utilizing the cage implant system.

Methods: Elasthane 80A (Medtronic, Minneapolis, MN), Silastic® (Dow Corning, Midland, MI), or Poly(ethylene terephthalate) (PET) (Toray Co., Tokyo, Japan) was implanted in 12 week old Sprague-Dawley rats using a subcutaneous cage implant system. Control rats were implanted with empty cages (n=4). Cages were implanted for 14 days. On days 4, 7, and 14 after implantation, exudates were collected and cells analyzed by flow cytometry for the following cell types: T cells (inclusive of cytotoxic (CD8+), helper (CD4+), and activated helper (CD4+/CD25+) subsets), B cells, granulocytes, and macrophages. After appropriate fluorescent antibody staining, cells were washed and fixed. Samples were then analyzed on Becton Dickinson Flow cytometer. Fluorescence positivity was determined by comparison to the isotype control. Volume and granularity distributions obtained by forward scatter and side scatter were used to separate cell populations. Material surfaces were removed on day 14 and stained with May Grunwald/Giemsa.

Results/Discussion: Total leukocyte concentration decreased over time for all groups. The total leukocyte concentration was material dependent as follows: Silastic>PET>Elasthane. No significant differences between biomaterials and empty cage controls were noted in exudative cell profiles at days 4, 7, and 14. No B cells were detected in any samples.

T cell concentrations increased slightly over the implant period for all material groups; however for the control group, T cell concentrations decreased over time. The Elasthane group had lower T cell concentrations than the Silastic and PET groups at all three timepoints (Figure 1). Analysis of T cell subsets showed that a higher percentage of helper T cells than cytotoxic T cells was present at all timepoints for all groups. At day 4, the Elasthane group had a higher percentage of CD25 (IL-2R α, T cell activation marker) expression on helper T cells than the Silastic and PET groups (Figure 2). At days 7 and 14, percent composition of T cells subsets was comparable between all groups.

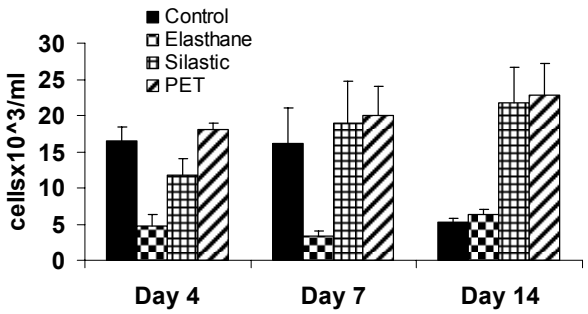

Figure 1 : T cell concentration for all groups at days 4, 7, and 14. Data represent the mean±SEM from 4 animals.

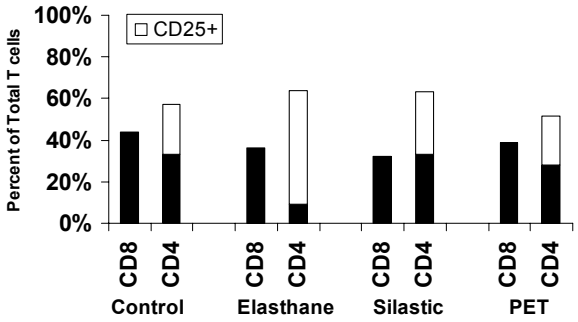

Figure 2: Percent Composition of T cell subsets from Day 4 exudates. Data represent the mean±SEM from 4 animals.

Despite these similarities in infiltrating T cell profiles, differences in cell adhesion/fusion were noted between material surfaces. At day 14, the Elasthane group had the highest adherent cell density followed by the Silastic group then the PET group. The percent fusion trend was reversed at day 14 (PET>Silastic>Elasthane).

Conclusions: Quantitative differences in exudate T cells and cell surface adhesion/fusion were identified in the Elasthane group compared to the Silastic and PET group. However, T cell subset composition was comparable between all groups. This study provides a platform for further T cell characterization at the biomaterial/tissue interface. These cell analyses coupled with cytokine/chemokine analyses are needed for elucidation of the T cell roles in the host response to biomaterials.

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