In Vivo Effect of Locally-Delivered Modulators on Cytokine Production and Macrophage Activation

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Statement of Purpose: Macrophages play an essential role in initiating the immune response resulting from the foreign body reaction to implanted biomaterials by producing anti-inflammatory cytokines and chemokines and eliminating tissue debris [1]. Macrophages are now believed to exist in a continuum of activation states that were formerly called M1 (classically activated state) and M2 (alternatively activated state) states [2]. The M2 state has also formerly been subdivided into M2a, M2b, and M2c phenotypes [3]. However, the current recommendations from the macrophage immunology community suggest not using these descriptions, but rather indicate the appropriate activator, e.g., M(IL-10), if IL-10 is used to activate macrophages[4]. This study aims to determine cytokine alterations in the presence of different modulators delivered at different time points after microdialysis probe implantation. The microdialysis sampling probe serves as both the implanted biomaterial as well as the means to both deliver modulators and collect cytokines. Using the microdialysis sampling technique enables each animal to serve as its own control since a control (no modulator) and treatment (modulator) are implanted into the same animal (Figure 1).

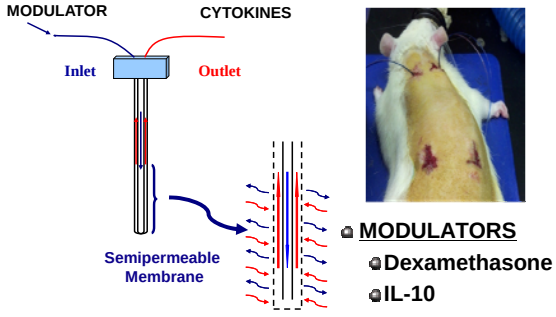

Figure 1. Overview of microdialyis implant approach with delivery and recovery.

Methods: Two microdialysis probes (CMA 20, Harvard Apparatus, Holliston, MA) were implanted in the dorsal subcutaneous space of male Sprague-Dawley rats. One probe was employed to be as a control probe and the second probe was employed as a treatment probe delivering either dexamethasone (20 µg/mL), M(Dex), or IL-10 (100 ng/mL), M(IL-10) with the same perfusion fluid used in the control side. IL-10 was determined to be biologically active via a cell-culture activated macrophage assay. After implantation, probes were flushed at 3 µL/min the rate was reduced to the desired flow rate of 1 µL/min. Dialysates were collected once an hr for 6 hr. After completion of the experiments, the rat was euthanized and the tissue surrounding the probes was excised for either immunohistochemical or qRT-PCR analysis. Cytokine concentrations were determined by either ELISA or multiplexed bead-based assays.

Results:
M(Dex) studies were performed by infusing the modulator at 3 days post implantation since we have previously shown CCL2 concentrations for daily infusions [5]. Interestingly, the CCL2 concentrations were about the same (range of 500-1500 pg/mL) in both probes on the first day of infusion, but significantly decreased for the M(Dex) probe treatment as the infusions continued through 6 days. While the CCL2 protein concentrations decreased, the qRT-PCR did not show a significant difference in the transcription between the M(Dex) and control probes.

For the M(IL-10) infusions, dialysate IL-6 concentrations were quantified with ranges between 70-200 pg/mL in both control and treatment animals. The concentrations are not statistically significant between the two treatment groups. More experiments need to be conducted with different cytokine measurements to draw conclusions for the effectiveness of IL-10 in modulating macrophages in this context.

Conclusions: As expected, dexamethasone clearly dampens the inflammatory response even when administered after the initiation of the foreign body reaction. However, IL-10 does not affect IL-6 and in previous work in our lab did not affect CCL2. Additional work is needed to determine if IL-10 localized infusion is sufficient to polarize macrophages at an implant site.

References:
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