In Vivo Measurement and Modulation of Cytokine Production in Rats

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Statement of Purpose: The temporal response of cytokines within a wound healing context is an important aspect to understand for improving biomaterials biocompatibility. In this work, four cytokines, interleukin-1β (IL-1β), IL-6, IL-10, and monocyte chemotactic protein-1 (MCP-1), were quantified from dialysates obtained from implanted microdialysis probes in rats. To determine if nitric oxide (NO) affected cytokine response, NO-releasing agents, S-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) and S-nitrosoglutathione (GSH-NO), were locally infused through implanted microdialysis sampling probes.

Methods: Microdialysis probes (CMA Microdialysis, North Chelmsford, MA) with a polyethersulfone (PES) membrane having molecular weight cut off (MWCO) of 100 kDa were implanted into the subcutaneous space of Sprague-Dawley rats. Six rats were used per group (18 rats) and each rat had two probes implanted into the dorsal subcutaneous space. A solution consisting of phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM sodium phosphate dibasic, 2 mM potassium phosphate monobasic, pH 7.4), 6% Dextran-70, and 0.1% bovine serum albumin (BSA) was passed through the probes to collect cytokines. An initial flow rate of 3 µL/min was used as a flush that was saved for analysis for the first 10 minutes. Then, the flow rate was 1 µL/min for 150 minutes with 30 minute intervals. Dialysates were collected on the day of implantation (day 0) and then 3 and 6 days post implantation. Cytokines in the dialysates were quantified using a bead-based immunoassay with a Luminex 100™ system.

In a separate set of animals (n=6 per group), Ringers solution was used as perfusion fluid. NO was delivered as 100 µM of SNAP or 100 µM of GSH-NO. Each sampling period was initiated with a 10-minute flush at 5 µL/min that was saved for analysis. The flow rate was then reduced to 1 µL/min and samples were collected every hour for three hours. Ringer's solution without an NO-releasing compound served as the control. The probes were perfused for 3 consecutive days. The dialysates were collected and MCP-1 was quantified using a standard ELISA kit.

Results: With the exception of IL-1β, all the targeted cytokines were collected and quantified on each of the sampling days. However, for many of the samples, cytokine levels below the detection limits of the analytical assay. IL-6 concentrations were the highest measured with an average of 920 pg/mL and was collected in 11 probes on the implantation day as shown in Figure 1 (1 probe failed, e.g., no fluid was obtained). IL-6 concentrations decreased on subsequent sampling days. Like IL-6, IL-10 concentrations also were highest on the day of implantation and subsequently decreased on the days post implantation. Interestingly, MCP-1 values were highest 3 days post implantation. IL-1β was not quantified in any of the dialysates collected immediately after implantation, and exhibited low concentrations near the detection limits of the assay on subsequent days.

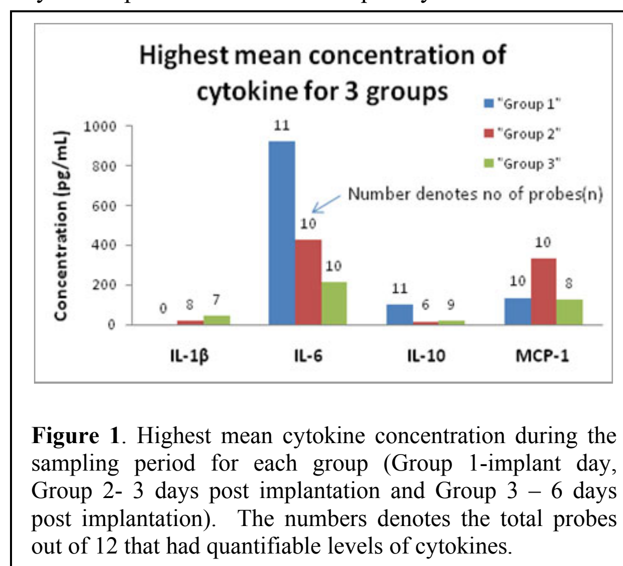

Figure 1. Highest mean cytokine concentration during the sampling period for each group (Group 1-implant day, Group 2- 3 days post implantation and Group 3 – 6 days post implantation). The numbers denotes the total probes out of 12 that had quantifiable levels of cytokines.

The MCP-1 concentrations were not significantly different between the SNAP and GSH-NO infusions vs. those obtained in controls. Typically, high concentrations of MCP-1 were collected within the ten minute flush periods on the second and third day.

Conclusions: Analysis of the in vivo cytokine profile in response to a foreign material is important for not only understanding, but more importantly for directing the host response to foreign objects. Both inflammatory and anti-inflammatory cytokines were observed. IL-10 is produced and is known to play a critical role in inhibiting the IL-1 family.[1,2] MCP-1 is required for the recruitment of leukocytes and macrophages at the wound site and was measured during each sampling day in this model system. The finding that NO has no effect on MCP-1 is in contrast to other findings showing MCP-1 expression to be lowered when NO is introduced into the local environment. [3,4]

References:

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