In Vivo Cytokine Sampling during the Foreign Body Response

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Statement of Purpose:

All implanted materials undergo a foreign body response that is believed to be principally driven by cytokine signaling produced by monocytes and macrophages. Obtaining quantitative information that includes the network of cytokines involved in the foreign body response as well as their concentrations is vital in order to understand and ultimately control the foreign body response at a tissue/biomaterial interface. Microdialysis sampling is an established *in vivo* technique that allows collection of analytes that can pass through the semi-permeable membrane. The purpose of this study is to illustrate both the promise and the challenges associated with using this analytical method to gain real-time and direct *in vivo* cytokine protein concentrations during a long term implant.

Methods:

Polyethersulfone microdialysis probes (length 10 mm, MWCO 100 kDa) and polyurethane tubes (length 25 mm, inner diameter 0.76 mm) from CMA Microdialysis were used. Microdialysis probes (2) were implanted at each side of dorsal subcutis of male Sprague-Dawley rats (270-275 g). Rats were given LPS (0.1 µg/g body weight, i.v) and dialysate samples were collected every 30 min for two hours. Cytokine concentrations (IL-6, IL-10, MCP-1, and TNF-α) were measured in 5 µl samples using a Rat Cytokine Linco*plex* Kit. Probes were calibrated before and after implantation with FITC-dextran (20 kDa).

To estimate cytokine concentrations produced during implantation, polyurethane tubes were implanted into the dorsal subcutis of rats. After three days, the tubes were explanted and the sample fluid was analyzed for cytokines.

Results / Discussion:

Figure 1 shows the cytokine collected in the dialysates from two probes for a three day survival-surgery experiment. Two hours after LPS addition, cytokine concentrations in dialysate from the acutely implanted probe reach 1470 pg/ml for IL-6, 462 pg/ml for MCP-1 and 65 pg/ml for IL-10. However, the probe implanted for three days the cytokine concentrations were lower and reached 453 pg/ml, 113 pg/ml and 85 pg/ml for IL-6, MCP-1, and IL-10. TNF-α was detected in the serum in these studies, but was not detected in dialysates.

To understand the nature of the decreased cytokine concentrations collected from the probe implanted for three days, the probes were calibrated *in vitro* before and after implantation using 20 kDa FITC-dextran. Table 1 shows that both probes exhibit similar recoveries suggesting tissue alterations affect the cytokine recovery differences noted in Figure 1.

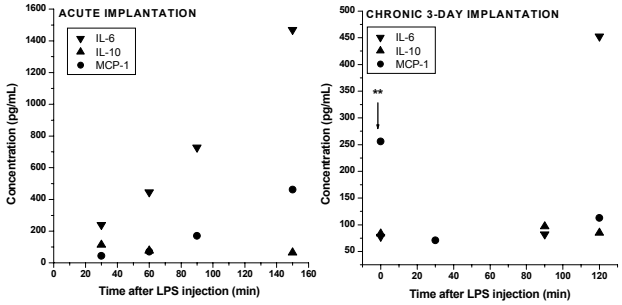

Figure 1. Cytokine concentrations from two probes. The symbols ** denotes cytokine concentrations in the dialysate prior to LPS injection.

Cytokine concentrations in the interstitial fluid collected from five polyurethane tubes are shown in Table 2. Three cytokines IL-10, MCP-1, and IL-6 are detectable in the interstitial fluid. Although the variations are relatively large which may be caused in part by some tubes having insufficient volume for analysis, the data shows that cytokines are in the extracellular fluid at detectable concentrations.

Table 1. FITC-dextran recovery (%) of the probes

Before Implantation		After Implantation	
1d	3d	1d	3d
3.82	5.44	3.54	4.31

Table 2. Cytokines from interstitial fluid inside the polyurethane tubes, pg/ml.

	IL-10	IL-6	MCP-1	TNF-α
1*	648	131	4920	N.D.
2	356	4630	10600	N.D.
3*	179	1010	4400	N.D.
4	N.D.	319	4010	N.D.
5	154	4760	14700	N.D.

N.D., not detected. * Indicates the volume collected was less than 5 µl needed for the assay.

Conclusions:

Cytokines are produced in detectable levels at an implant site. Microdialysis sampling has promise to be used as an analytical tool to sample cytokines.

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