In Vitro Cytokine-Associated Immune Response to Common Biomaterials

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Statement of Purpose: Inflammation has long been implicated in the failure of an array of implants[1]. Cytokines, many of which can be categorized as pro- or anti-inflammatory, are produced by macrophages at the surface of an implanted biomaterial[2]. Biomaterial surface properties dictate the types and amounts of cytokines produced by adherent macrophages. These macrophage-derived cytokines dictate the actions of immune cells in the tissue surrounding an implant. An in vitro technique has been implemented to investigate cytokine production from activated monocytes cultured on the surface of common biomaterials.

Methods: Human monocytes (THP-1) were activated via 24 hours of incubation with 1μg/mL lipopolysaccharide (LPS, endotoxin). Supernatant was collected from LPS-activated monocytes and analyzed for MCP-1, TNF-α, MIP-1α, IL-6, IL-8, IL-1β, IL-10, and IL-1ra using a Luminex bead array (BioRad). Activated monocytes were seeded onto sterilized discs (1cm^2) of polyethylene (PE), polyurethane (PU), polymethylmethacrylate (PMMA), expanded polytetrafluoroethylene (ePTFE), organo-tin stabilized polyvinyl chloride (PVC), or directly onto tissue-culture polystyrene (TCPS). Viable cells were counted and supernatants were collected 24 and 48 hours after seeding and analyzed for cytokine content as described above. Material-induced cytokine production was normalized to LPS-induced cytokine production for comparison between biomaterials.

Results/Discussion: Normalized cytokine production for each material is shown in Figure 1.

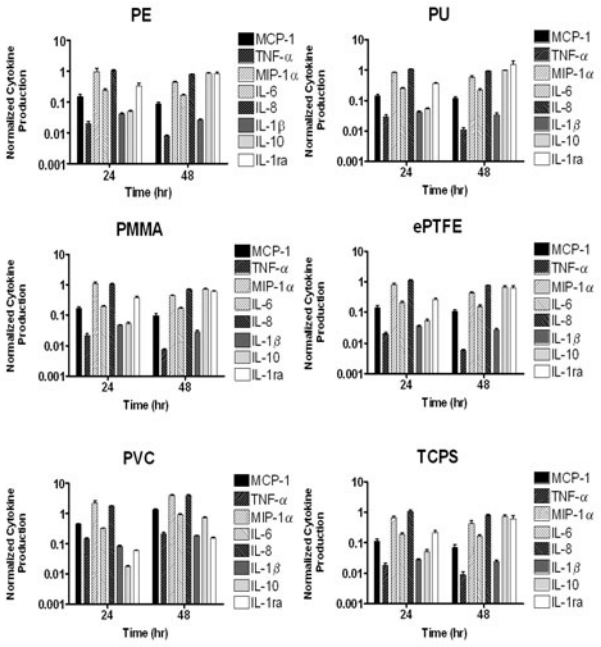

Figure 1. *Cytokine production from activated monocytes seeded onto biomaterial surfaces.*

Monocyte production of pro-inflammatory cytokines (MCP-1, TNF-α, MIP-1α, IL-6, IL-8, and IL-1β) is similar at 24 and 48 hours for PE, PU, PMMA, ePTFE, and TCPS. However, in comparison to these materials, pro-inflammatory cytokine production was higher for organo-tin PVC. In addition, monocyte production of IL-1ra, an anti-inflammatory cytokine, was similar for PE, PU, PMMA, ePTFE, and TCPS, but lower for organo-tin PVC. However, the production of IL-10, another anti-inflammatory cytokine, was not significantly different for any of the materials tested. Table 1 summarizes the mean cytokine production for each of the biomaterials after 48 hours, with the largest values bolded and underlined.

	PE	PU	ePTFE	PMMA	PVC	TCPS
MCP-1	0.09	0.1	0.1	0.1	**1.4**	0.07
TNF-α	0.008	0.01	0.006	0.007	**0.2**	0.009
MIP-1α	0.4	0.6	0.4	0.4	**4.0**	0.4
IL-6	0.2	0.2	0.2	0.2	**0.9**	0.2
IL-8	0.8	0.9	0.7	0.7	**4.1**	0.8
IL-1β	0.03	0.03	0.03	0.03	**0.2**	0.02
IL-10	0.8	1.0	0.7	0.7	0.7	0.7
IL-1ra	0.8	**1.6**	0.6	0.6	0.2	0.6

Table 1. *Mean cytokine production from activated monocytes seeded onto biomaterial surfaces (48 hours).*

Organo-tin stabilized PVC, a highly cytotoxic material, induces increased monocyte production of pro-inflammatory cytokines (4-20 fold) and decreased production of anti-inflammatory cytokines (8-fold) as compared to standard biomaterials. This pattern of cytokine production may indicate increased inflammation associated with implanted organo-tin stabilized PVC. The cytokine production profiles of PE, PU, PMMA, ePTFE, and TCPS would be reflective of a lesser degree of inflammation induced by these materials. This result is not surprising since these materials are commonly used in implanted devices or as cell culture substrates.

Conclusions: An in vitro technique for determining cytokine production from monocytes seeded onto biomaterials has been developed. Cytotoxic organo-tin stabilized PVC induced a more pro-inflammatory cytokine profile, while cytokine profiles induced by common biomaterials were similar and less pro-inflammatory. Cytokine production from macrophages seeded onto material surfaces may be an indication of the degree of inflammation induced by that material.

References:

1. Tang, LP. Am J Clin Pathol. 1995;103:466-471.
2. Brodbeck, WG. J Biomed Mater Res. 2003;64A:320-329.