Immune Cell Response to Anti-inflammatory Cytokine Tethered Non-fouling Surfaces

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Introduction: The objective of this research is to develop an anti-inflammatory and immune-suppressive cytokine immobilized non-fouling surface that would (1) eliminate all adventitious adsorption of protein in a robust manner, and (2) facilitate specific interactions with immune cells around the implant for both attenuating inflammation and promoting wound healing response. Cytokines considered being anti-inflammatory and immune suppressive are IL-1ra, TGF-β1, IL-10 and IL-4 [1]. As a proof of concept of the cellular effect to the cytokine tethered surfaces, the treated and the untreated surfaces have been investigated.

Methods: IL-1ra for surface attachment and soluble treatment was expressed as a fusion protein with elastin-like polypeptide (ELP) by bacterial expression as a fusion protein in *e. coli* as described by Betre [2]. Alkane-SAM (Self-Assembled-Monolayer) was generated on Au coatings and IL-1ra and BSA was immobilized using EDC-NHS. THP-1 human monocytes were cultured on the above treated surface at 10^5 cells/well in 6 ml of media. Untreated and lipopolysaccharide (LPS)-stimulated at 1 μg/ml monocytes were cultured on three surfaces: SAM only, immobilized IL-1ra on the SAM, and SAM with soluble IL-1ra. Supernatants collected from each condition and time point was analyzed using multiplexed immuno-fluorescent assays.

Results/Discussion: Figure 1 shows THP-1 human monocytes cultured on the SAM surface, 72 hrs after seeding, (a) without LPS, (d) with LPS stimulation (100 ng/ml), (c) SAM with soluble IL-1ra, (b) SAM with immobilized IL-1ra.

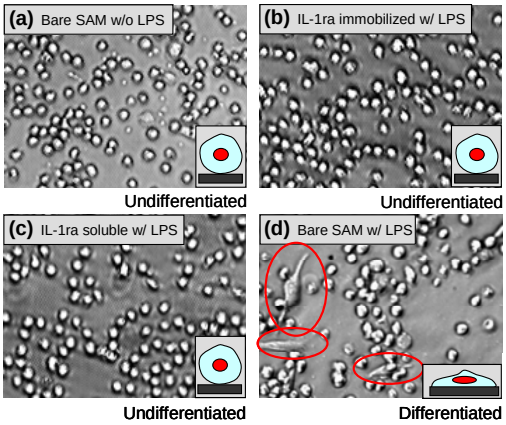

Figure 1. Optical images of human monocytes.

Cell density of THP-1 on the SAM surface without LPS stimulation (a) was higher than LPS stimulation (d) and some of monocytes were found to be differentiated under LPS stimulation, which has been found to be the characteristics of the THP-1 monocytes under LPS stimulation. Note that both soluble and immobilized IL-1ra (Figure (a) and (b)) inhibit the differentiation of THP-1 monocytes by LPS stimulation, indicating that both the soluble and the immobilized IL-1ra are bioactive. Figure 2 shows 6 cytokines sampled from supernatant after exposing human monocytes to bare SAM and SAM with soluble or immobilized IL-1ra. Expression of the pro-inflammatory cytokines, IL-8, TNF-α and MIP-1α on SAM+LPS were increased significantly compared to the SAM, indicating LPS stimulation of the monocytes. However, even with LPS stimulation, pro-inflammatory cytokine expression was significantly attenuated while pro-wound healing cytokine expression was increased. The difference of cytokine expression between immobilized and soluble IL-1ra was not significant. This data shows that the immobilized IL-1ra is as bioactive as the soluble IL-1ra.

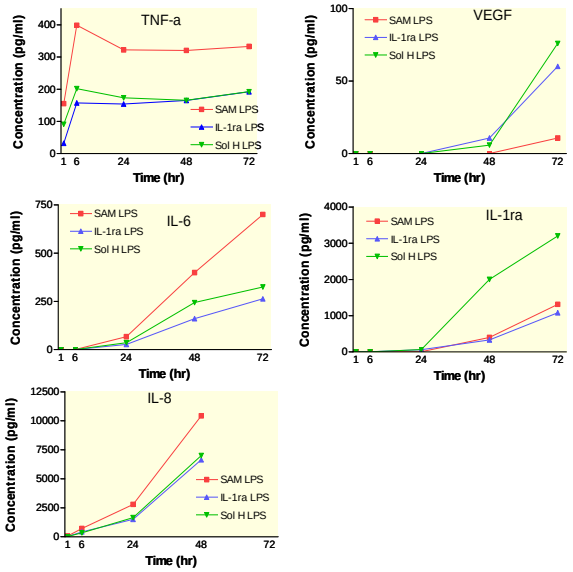

Figure 2. Temporal profile of cytokines secreted from THP-1 human monocytes.

Conclusions

We have demonstrated that IL-1ra-tethered non-fouling surface using thiol-SAMs imparts anti-inflammatory behavior on human monocytes. The immobilized IL-1ra on SAM surface maintained it's bioactivity up to 72hrs and reduced the pro-inflammatory cytokine expression indicating attenuated inflammation and promoted wound healing cytokines.

References

1. Thomson AW et. al, The cytokine handbook. 4 ed. 2003, San Diego: Academicpress.
2. Bertre H, Controlled intra-articular drug delivery system based on thermally responsive biopolymers, Ph. D thesis in Biomedical Engineering. 2005, Duke university: Durham.