Targeting the SIRPα1 Signaling Pathway to Reduce the Inflammatory Response to Polymeric Surfaces

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Statement of Purpose: Clinical procedures often require whole blood exposure to polymeric surfaces such as poly vinyl chloride (PVC) and polyurethane (PU). Proteins present in the serum adsorb on the synthetic surface, creating an environment conducive to inflammatory cell attachment. Polymorphonuclear lymphocytes (PMNs) and platelets attach to the polymer surface, which results in an inflammatory response characterized by the release of pro-inflammatory cytokines[1]. This inflammatory response can lead to increased clinical complications post-surgery. For example, post-surgical complications following cardiopulmonary bypass (CPB) are associated with an acute and systemic inflammatory response that contributes to neurocognitive issues and dysfunction of the cardiac, renal, and hepatic systems[2]. Addressing the inflammatory response that is a result of aberrant blood-material interactions remains an unmet need in cardiovascular biomaterials. We have developed an anti-inflammatory strategy that targets signal regulatory protein alpha 1 (SIRPα1). SIRPα1 is a member of the immunoreceptor tyrosine-base inhibition motif (ITIM) family of expressing molecules. When SIRPα1 binds to its cognate ligand, CD47, signal transduction events mediated by tyrosine phosphorylation of the ITIM domain of SIRPα1, initiate cytoskeletal depolymerization and dephosphorylation of Myosin IIa[3]. Our laboratory has found the CD47-SIRPα1 signaling pathway to be involved in the prevention of cellular adhesion to polymeric surfaces. We test here the hypothesis that targeting SIRPα1 can prevent cellular adhesion in *in vitro* and *ex vivo* systems. Cellular adhesion assays were used to analyze anti-SIRPα1 antibody modified polymeric surfaces in comparison with unmodified materials. In addition, we utilized biochemical methods to monitor changes in intracellular signaling.

Materials & Methods: Cellular adhesion assays: THP-1 or HL-60 cells were differentiated using phorbol 12-myristate 13-acetate (PMA) and allowed to attach to PVC or PU 1x1 cm films. PVC or PU films were either unmodified, coated with avidin using photoactivatable polymer techniques, or avidin and biotin-conjugated antibodies (anti-SIRPα1 extracellular (SE7C2), intracellular (C20) or isotype controls mouse IgG and goat IgG, respectively). Cell adhesion was quantified after the cells were fixed with 2% paraformaldehyde and stained with DAPI for 30 minutes to allow for counting using fluorescent microscopy. Immunoprecipitation (IP) assays: HL-60 cells were differentiated with PMA for 24 hours and either left untreated or treated with 10 µg anti-SIRPα1 antibody for an additional 1 hour. Lysates were then incubated with anti-SIRPα1 antibody conjugated to affinity beads. IPs were resolved on an SDS gel, transferred to PVDF membrane and probed for anti-phosphotyrosine levels of the SIRPα1 and Myosin IIa proteins. As a control for uniform protein loading, western blots were also probed for anti-SIRPα1 or anti-Myosin IIa.

Results: The cellular adhesion assays demonstrated significantly decreased cellular attachment to the SE7C2 anti-SIRPα1 antibody coated PVC and PU films when compared to the unmodified films. For the unmodified PVC, the average number of attached cells was 153.8 (+/- 11.6) versus 18.6 (+/- 3.1) for the anti-SIRPα1 antibody coated PVC. Similarly, the PU films had 58 (+/- 4.5) cells on the unmodified compared to 12.4 (+/- 2.7) on the antibody coated films. For both PVC and PU, the P value was <0.001 indicating the changes are significant. Although avidin-coated surfaces revealed slightly less cell attachment compared to the unmodified surfaces, the difference was not significant. Immunoprecipitation assays demonstrated over 2-fold increased tyrosine-phosphorylation of SIRPα1 following cell culture treatment with anti-SIRPα1 antibody suggesting the antibody can act as an agonist for the SIRPα1 receptor and initiate signal transduction. Additional studies revealed over 2-fold change in tyrosine phosphorylation of myosin IIa when HL-60 cells were treated with the anti-SIRPα1 antibody when compared to the untreated control.

Conclusions: Functionalizing polymeric surfaces with antibodies specific for the extracellular domain of SIRPα1 can inhibit inflammatory cell interactions with synthetic surfaces. The anti-SIRPα1 antibody appears to act as an agonist for the SIRPα1 receptor and initiated a signal transduction cascade as evident from the phosphorylation of SIRPα1 and myosin IIa.

References: [1](Levy JH. et al. Ann Thorac Surg. 2003;75:S715-S720.) [2](Ishibashi N. et al. J Thorac Cardiovas Surg. 2010.) [3](Tsai RK. et al. JBC 2008; 180:989-1003.)

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