Influence of Smooth Muscle Cell Phenotype on Endothelial Cell Response to Biomaterial-Pretreated Leukocytes in an EC/SMC Co-Culture Model

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Introduction: Endothelialized synthetic vascular grafts suffer from difficulties in endothelial cell (EC) retention, and tissue engineered vascular constructs that mimic nature are early in their development. Also, stents can fail due to intimal hyperplasia, and themselves cause an inflammatory and thrombotic response. Central to advancing these strategies is an understanding of EC/blood/ biomaterial interactions. Biomaterial activated monocytes were previously shown to induce a proinflammatory EC phenotype following static co-culture[i], and biomaterial-activated monocytes and PMNs differentially induced a procoagulant EC phenotype following static co-culture[ii]. The objective of this research is to elucidate the influence of biomaterial-induced activation of LKs on the EC response in the EC/smooth muscle cell (SMC) cross-talk in a co-culture system. It is hypothesized that treatment of ECs with biomaterial-activated LKs will result in a proinflammatory and procoagulant EC phenotype, and that this induced phenotype will be modulated by SMC presence and phenotype - inhibited by contractile SMC phenotype.

Materials and Methods: Polyethylene teraphalate (PET) transwell filters (4.2 cm^2, 0.4μm pores, BD Biosciences) were incubated in MCDB 131 (Mediatech Cellgro) with 5% (v/v) FBS containing 1% (v/v) HyQ Pen-Strep, 1% (v/v) L-glutamine, 2ng/mL hFGF-b (PeproTech), 0.5 ng/ml hEGF (Gibco) (secretory media) overnight to enhance cell attachment. Filters were then inverted and 400μl of human aortic SMCs (HASMC; Clonetics, Walkersville, MD) (0, 15000, or 90000 cells/cm^2) were dripped onto filter and allowed to adhere (2h, 37°C). Filters were returned to their wells and covered in secretory media. *Secretory phenotype*: Human aortic ECs (HAEC; Clonetics) (40,000 cells/cm^2) were added to the inner compartment, and plates were incubated (48 h, 37°C) prior to assay. *Contractile phenotype*: HASMCs were incubated overnight in secretory media. Media was then replaced with secretory media lacking FBS or any growth factors for 48h to induce contractility. Then, media was replaced with secretory media containing only 2% FBS (contractile co-culture media). HAEC (40,000 cells/cm^2) was added to the inner compartment, and plates were incubated (48 h, 37°C) prior to assay. Peripheral human whole blood was collected and monocytes and PMNs were isolated with CD14 and CD15 positive selection (Miltenyi Biotec), respectively. Isolated monocytes or PMNs were mixed with PS (45 μm, Polysciences, Inc) beads at 5.4*10^4 beads/ml (2 hrs). For LK, untreated LKs acted as the negative time control, 10μM fMLP treatment acted as the positive control. For ECs, TNF-α or IL-1β treatment served as the positive control. The LK/bead mixtures and controls were applied to the EC surface of the EC/SMC co-cultures for 5 or 24h, depending on the marker assessed. For flow cytometry, proinflammatory /pro-anticoagulant markers included ICAM-1 (R6.5), VCAM-1 (1.G1b1; Southern Biotechnology Associates), and E-selectin (BBIG-A5; R&D Systems), tissue factor (TF; HTF-1) and thrombomodulin (1A4, BD Biosciences). Mean fluorescence intensity was determined using a BD LSR flow cytometer.

Results: ELISAs for IL-8 and MCP-1 and immuno-histochemistry for α-smooth muscle actin were used to verify desired SMC phenotype for our two co-culture methods. *Flow cytometry*: Proinflammatory markers E-selectin, ICAM-1, and VCAM-1 were upregulated on ECs (in co-culture with "secretory" or "contractile" SMCs) following incubation with fMLP or biomaterial-pretreated monocytes (but not PMNs). Their expression was further increased with increasing number of "secretory" SMCs. Alternately, E-selectin expression was suppressed by increasing number of "contractile" SMCs, ICAM-1 expression was not influenced by the presence of "contractile" SMCs, and VCAM-1 expression was increased by increasing number of "contractile" SMCs. Procoagulant marker TF was upregulated on ECs (in co-culture with varying concentrations of "secretory" or "contractile" SMCs) following incubation with fMLP or biomaterial-pretreated monocytes (but not PMNs). Similar to E-selectin, TF expression was further increased with increasing number of "secretory" SMCs, but alternately suppressed by increasing number of "contractile" SMCs. Anti-coagulant marker thrombomodulin was downregulated on ECs (in co-culture with "secretory" or "contractile" SMCs) following incubation with fMLP or biomaterial-pretreated monocytes *or* PMNs. This downregulation was suppressed with increasing number of both "secretory" and "contractile" SMCs.

Conclusions: These studies show the ability to control the phenotypic state of cultured HASMCs using specific co-culture methods. The presence of "secretory" SMCs enhanced EC activation in response to biomaterial-pretreated monocytes as exemplified by increases in E-selectin, ICAM-1, VCAM-1 and TF expression. Alternately, the presence of "contractile" SMCs suppressed EC activation in response to biomaterial-pretreated monocytes (and PMNs to a small degree) as exemplified by subdued E-selectin, TF, and thrombomodulin expression.

***Acknowledgements*:** Funded by a Whitaker Biomedical Engineering Research Grants Transitional Funding

References:

[i] EA Lester & JE Babensee, J. Biomed. Mater. Res., 64A: 397 (2003).
[ii]SL Rose & JE Babensee. J. Biomed Mater. Res. 72A:269 (2005).