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**Whitaker Biomedical Engineering Research Grants Transitional Funding
Functional Consequences of Activated Leukocytes in Tissue Engineering**

Final Report

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ABSTRACT

Cardiovascular devices continue to fail due to the lack of non-thrombogenic biomaterials. Endothelialized synthetic vascular grafts suffer from difficulties in endothelial cell (EC) retention. Tissue engineered vascular constructs that mimic nature are early in their development. Vascularization of complex, three-dimensional tissue engineered constructs is critical to their function. Central to advancing these strategies is an understanding of EC/blood/biomaterial interactions. Cardiovascular disease is the leading cause of death in the United States. Coronary artery stent placement has been a significant advance in the percutaneous treatment of atherosclerotic disease, but in-stent restenosis remains a fundamental limitation. While animal models can provide insight in the pathophysiology of in-stent restenosis, elucidation of the relative importance of the various components of the stent and the hemodynamic factors on this process and the molecular factors can be difficult. For this purpose, a model system will be developed to analyze the relative roles of biomaterial-induced activation of leukocytes on EC proliferation and phenotype in the crosstalk between ECs and SMCs in a co-culture system under static and dynamic conditions. In previous work, we have demonstrated that biomaterial activated leukocytes induce a proinflammatory and procoagulant phenotype on human umbilical vein endothelial cells (HUVECs) following static co-culture. Preliminary studies have shown that in human aortic EC (HAEC) and human aortic SMC (HASMC) co-culture, HASMC phenotype modulates HAEC response to biomaterial-pretreated leukocytes. More specifically, the presence of secretory SMCs amplifies the proinflammatory/procoagulant phenotype, while the presence of contractile SMCs suppresses this phenotype. The overall hypothesis of this work is that biomaterial contact by leukocytes affects downstream healing responses. The specific goal of this research is to elucidate the influence of biomaterial-induced activation of leukocytes on ECs in an EC/SMC co-culture system.

In a quiescent native vessel, SMCs are contractile. However, during injury and wound healing, they take on a proliferative and secretory phenotype. Thus, it is important to examine the impact of both contractile and secretory SMCs on EC interactions with biomaterial-pretreated leukocytes to best model vascular healing *in vitro*. Two co-culture methods were developed to obtain more secretory or contractile SMC phenotypes. To characterize the resulting phenotypes, ELISAs were performed for secreted interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) and immunohistochemistry for α -smooth muscle actin. Forced contractile HASMCs demonstrated organized α -smooth muscle actin filaments, minimal interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) secretion, and low intracellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and tissue factor expression. Organization of α -smooth muscle actin was lost in more secretory HASMCs in co-culture, and IL-8 and MCP-1 secretion, as well as ICAM-1, VCAM-1, and tissue factor expression were significantly upregulated at both time points. Alternately, less secretory HASMCs in co-culture showed similar characteristics to forced contractile HASMCs at the 48h time point, while by the 72h time point they behaved similarly to more secretory HASMCs.

These co-culture systems could be useful in better understanding vascular healing, however there remain time constraint considerations for maintaining culture integrity/cell phenotype.

Flow cytometry for proinflammatory and pro/anti-coagulant markers on ECs (in co-culture with varying concentrations of “secretory” or “contractile” SMCs) following incubation with biomaterial-pretreated leukocytes was performed. 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) above the untreated monocyte control. For all three proinflammatory markers, their expression was further increased with increasing number of “secretory” SMCs in co-culture. However, each marker showed a different trend when in co-culture with “contractile” SMCs. E-selectin expression was suppressed by increasing number of “contractile” SMCs in co-culture, ICAM-1 expression was not influenced by the presence of “contractile” SMCs, and VCAM-1 expression retained the same increasing trend, where increasing number of “contractile” SMCs further increased VCAM-1 expression. Procoagulant marker tissue factor 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) above the untreated monocyte control. Similar to E-selectin expression, tissue factor expression was further increased with increasing number of “secretory” SMCs in co-culture, but alternately suppressed by increasing number of “contractile” SMCs in co-culture. Finally, anti-coagulant marker thrombomodulin was downregulated on ECs (in co-culture with “secretory” or “contractile” SMCs) following incubation with fMLP or biomaterial-pretreated monocytes *and* PMNs below the untreated monocyte or PMN control. This downregulation was suppressed with increasing number of both “secretory” and “contractile” SMCs.

Limitation of EC proliferation *in vivo* can lead to prolonged endothelial denudation or the inability to maintain an EC monolayer on vascular grafts. To examine the proliferative status of HAECs in co-culture with more secretory (5% FBS) or less secretory (2% FBS) HASMCs following incubation of the HAEC surface with biomaterial-pretreated monocytes or PMNs, the same experimental setup was used as described previously for flow cytometry, with the addition of BrdU for the final 45 minutes of culture. It was found that treatment of monocultured HAECs incubated with TNF- α , as well as fMLP or biomaterial-pretreated monocytes, and not PMNs significantly decreased HAEC proliferation in both co-culture systems. In addition, the presence of more secretory HASMCs reduced HAEC proliferation for HAECs incubated with media alone, untreated monocytes (dose dependent in these cases), or fMLP monocytes, while the presence of less secretory HASMCs had no effect on HAEC proliferation. Because the presence of more secretory HASMCs significantly reduces HAEC proliferation, further decreases due to TNF- α , or fMLP or biomaterial-pretreated monocytes were indiscernible in the co-cultures.

In the case of endothelial denudation, underlying vascular SMCs interact directly with blood cells. To study the effect of biomaterial-pretreated leukocytes directly on HASMC phenotype, untreated, fMLP-pretreated, or biomaterial-pretreated monocytes or PMNs were applied to more secretory (5% FBS) or forced contractile (serum-free and growth factor-free) HASMC monocultures for a 5h or 24h incubations. For more secretory HASMCs (5% FBS), 5h treatment with positive controls (TNF- α for pro/anti-coagulant markers, IL-1 β for proinflammatory markers), fMLP-pretreated or biomaterial-pretreated monocytes or PMNs significantly upregulated ICAM-1 and tissue factor expression above the untreated control. Incubation with fMLP-pretreated monocytes upregulated ICAM-1 and tissue factor expression above incubation with untreated monocytes. However, treatment of more secretory HASMCs

with positive controls, fMLP-pretreated or biomaterial-pretreated leukocytes did not affect VCAM-1 or thrombomodulin expression. These trends were maintained at the 24h incubation, thus data has been omitted for clarity. For the 5h incubation begun with forced contractile HASMCs (serum-free and growth factor-free), treatment with positive controls upregulated ICAM-1 and tissue factor expression above the untreated media control. Also, fMLP- or biomaterial-pretreated monocytes or PMNs significantly upregulated ICAM-1, tissue factor, and VCAM-1 expression above the untreated media control. Furthermore, biomaterial-pretreated monocytes significantly upregulated ICAM-1, VCAM-1 and tissue factor expression above untreated monocytes. Additionally, fMLP-pretreated monocytes upregulated tissue factor expression above untreated monocytes. Lastly, fMLP pretreated PMNs upregulated ICAM-1 expression above untreated PMNs. For all treatments and markers examined at the 5h time point, expression was significantly lower than with more secretory HASMCs. For the 24h time point, with forced contractile HASMCs, treatment with positive controls upregulated VCAM-1, ICAM-1, and tissue factor expression above the media control. Additionally, fMLP- and biomaterial-pretreated monocytes or PMNs, as well as *untreated* monocytes or PMNs significantly upregulated ICAM-1, tissue factor, and VCAM-1 expression above the media control. However, fMLP- or biomaterial- pretreated monocytes further upregulated VCAM-1 and ICAM-1 expression above incubation with untreated monocytes. For the untreated control, VCAM-1 expression was significantly higher at the 24h time point as compared to the 5h time point. In addition, for all treatments, VCAM-1, ICAM-1, or tissue factor expression was often significantly higher at the 24h time point as compared to the 5h time point.

In these studies we have shown the ability to control the phenotypic state of cultured HASMCs based on specific co-culture methods. The presence of “secretory” SMCs in co-culture enhanced EC activation in response to biomaterial-pretreated monocytes as exemplified by increases in E-selectin, ICAM-1, VCAM-1, and tissue factor expression. Alternately, the presence of “contractile” SMCs in co-culture suppressed EC activation in response to biomaterial-pretreated monocytes (and PMNs to a small degree) as exemplified by subdued E-selectin, tissue factor, and thrombomodulin expression. Direct incubation of biomaterial-pretreated monocytes or neutrophils with more secretory HASMCs further increased HASMC ICAM-1 and tissue factor expression. Direct incubation of biomaterial-pretreated monocytes or neutrophils with forced contractile HASMCs upregulated ICAM-1, VCAM-1, and tissue factor expression above the presence of serum-containing media alone.

PUBLICATIONS

Rose, S.L., Babensee, J.E., Phenotype of endothelial cells after co-culture with biomaterial-treated whole blood, Journal of Biomaterial Science, Polymer Edition, submitted.

Rose, S.L., Babensee, J.E., Characterization of Endothelial Cell/Smooth Muscle Cell Co-Culture Systems Resulting in Alternate Smooth Muscle Cell Phenotypes, Annals of Biomedical Engineering, submitted.

Rose, S.L. and Babensee, J.E., Smooth Muscle Cell Phenotype Alters Co-Cultured Endothelial Cell Response to Biomaterial-Pretreated Leukocytes, Journal of Biomedical Material Research, submitted.

Rose, S.L., Babensee, J.E., Procoagulant phenotype of endothelial cells after co-culture with biomaterial-treated blood cells, Journal of Biomedical Materials Research, 72A: 269-278 (2005).

Lester, E.A., Babensee, J.E., Proinflammatory phenotype of endothelial cells after co-culture with biomaterial-treated blood cells, Recipient of the 2002 Society for Biomaterials *Student Award for Outstanding Research-Undergraduate, Masters or Health Science*, Journal of Biomedical Material Research, 64A: 397-410 (2003).

PRESENTATIONS

Rose, S.L. and Babensee, J.E., In *vitro* Model of Vascular Healing in the Presence of Biomaterials 32nd Annual Meeting of the Society for Biomaterials, Chicago, IL, April 18-21, 2007. (submitted)

Rose, S.L., Babensee, J.E., In Co-culture, Smooth Muscle Phenotype Alters Endothelial Response to Biomaterial-Treated Leukocytes, 2006 Annual Meeting of the Biomedical Engineering Society, Chicago, IL, October 11-12, 2006.

Rose, S.L., Babensee, J.E., Influence of Smooth Muscle Cell Phenotype on Endothelial Cell Response to Biomaterial-Pretreated Leukocytes in an EC/SMC Co-Culture Model, 31th Annual Meeting of the Society for Biomaterials, Pittsburgh, PN, April 26-29, 2006.

Rose, S.L., Babensee, J.E., In vitro model of vascular healing in the presence of biomaterials: endothelial cell response, 2005 Annual Meeting of the Biomedical Engineering Society, Baltimore, MD, September 28 – October 1, 2005.

Rose, S.L., Babensee, J.E., Endothelial Cell Procoagulant Phenotype Upon Co-Culture With Biomaterial-Treated Blood Cells, 2004 Annual Meeting of the Biomedical Engineering Society, Philadelphia, PN, October 13-16, (2004).

Rose, S.L., Babensee, J.E., Procoagulant Phenotype of Endothelial Cells After Co-culture with Biomaterial-Treated Whole Blood, 7th World Biomaterials Congress, Sydney, Australia, May 17-21, (2004).

Babensee, J.E., Functional consequences of activated leukocytes in tissue engineering, 2003 Whitaker Foundation Biomedical Engineering Research Conference, Hilton La Jolla Torrey Pines, La Jolla, CA, August 7-10, (2003).

Lester, E., Babensee, J., Proinflammatory phenotype of endothelial cells after co-culture with biomaterial-treated blood cells, 28th Annual Meeting of the Society for Biomaterials, Tampa, Florida, April 24-27, (2002).

Lester, E.A., Babensee, J.E., Endothelial cell phenotype induced by biomaterial-activated blood cells, American Institute of Chemical Engineers Annual Meeting, Reno, Nevada, USA, November 4-9, (2001).

Lester, E.A., Babensee, J.E., Role of biomaterial-activated monocytes in mediating endothelial cell proinflammatory phenotype, 2001 Annual Meeting of the Biomedical Engineering Society, Sheraton Imperial Hotel, Durham, NC, October 4-7, (2001).

Lester, E.A., Patel, S.R., Babensee, J.E., Consequence of blood cell activation by biomaterials on endothelial cell phenotype, 27th Annual Meeting of the Society for Biomaterials, Saint Paul, Minnesota, April 24-29, (2001).

Lester, E.A., Patel, S.R., Babensee, J.E., Consequence of blood cell activation by biomaterials on endothelial cell phenotype, 5th Annual Hilton Head Workshop & Annual ET Workshop, Sea Pines Plantation, Hilton Head Island, SC, February 21-25, (2001).

FUNDING

GTEC Research Seed Grant: National Science Foundation Georgia Tech/Emory Center for the Engineering of Living Tissues

Project Title: Mechanisms of Immune Responses Towards Antigens from Tissue Engineered Devices

Dates of Project: May 15, 2000 – May 14, 2001

Funded budget: \$33,500

Goal: Adjuvanticity of biomaterials in tissue engineering in the enhancement of immune responses to associated antigens (Antibody generation and T cell responses to a model antigen delivered with biomaterial scaffolds, microparticles and encapsulated cells)

Georgia Research Alliance Matching Funds for GTEC

Project Title: Mechanisms of Immune Responses Towards Antigens from Tissue Engineered Devices

Funded budget: equipment funds of \$11,295

Emory/Georgia Tech Biomedical Technology Research Center Seed Grant: Emory/Georgia Tech Biomedical Technology Research Center

Co-investigator: Ifor Williams, MD/Ph.D., Emory University

Project Title: Oral DNA Vaccines Incorporating Chitosan Nanoparticles for Induction of Mucosal Immunity

Dates of Project: July 1, 2000 – June 30, 2001

Funded budget: \$27,000

Goal: Biomaterial nanoparticles for DNA vaccines and gene therapy (Chitosan nanoparticles encapsulating DNA for induction of mucosal immunity)

Wallace H. Coulter Translational/Clinical Research Seed Grant Program: The Coulter Foundation

Co-investigator: Ifor Williams, MD/PhD, Emory University

Project Title: Enhancing the efficacy of DNA vaccines via encapsulation in chitosan nanoparticles: Studies in a preclinical mouse model and translational studies using human dendritic cells

Dates of Project: July 1, 2001 – June 30, 2002

Funded budget: \$100,000

Goal: Biomaterial nanoparticles for DNA vaccines and gene therapy (Chitosan nanoparticles encapsulating DNA for induction of mucosal immunity)

Whitaker Biomedical Engineering Research Grants: The Whitaker Foundation

Project Title: Functional Consequences of Activated Leukocytes in Tissue Engineering

Dates of Project: September 1, 2001 – August 31, 2004

Funded budget: \$226,175

Goal: Cellular and molecular aspects of inflammatory responses in the context of tissue engineering. (Biomaterial induced monocyte, neutrophil and platelet activation, their microparticles and endothelial cell activation (proinflammatory and coagulant phenotype) under static and dynamic conditions)

GTEC Research Grant: National Science Foundation Georgia Tech/Emory Center for the Engineering of Living Tissues

Project Title: Mechanisms of Immune Responses Towards Antigens from Tissue Engineered Devices

Dates of Project: September 1, 2001 – August 31, 2002

Funded budget: \$51,308

Goal: Adjuvanticity of biomaterials in tissue engineering in the enhancement of immune responses to associated antigens (Antibody generation and T cell responses to a model antigen delivered with biomaterial scaffolds, microparticles and encapsulated cells)

Arthritis Investigator Award: The Arthritis Foundation

Project Title: Tissue Engineering for Rheumatoid Arthritis: A Biomaterial-Centered Approach for Controlling Dendritic Cell Phenotype

Recipient of *Hulda Irene Duggan Arthritis Investigators Award* for top four review scores

Dates of Project: July 1, 2002 – June 30, 2005

Funded budget: \$222,000

Goal: Tissue engineering for rheumatoid arthritis (RA) (Identification of biomaterials for cartilage and bone tissue engineering that inhibit DC maturation with in vivo justification in RA animal models)

Wallace H. Coulter Translational/Clinical Research Seed Grant Program: The Coulter Foundation

Co-investigator: Ifor Williams, MD/PhD, Emory University

Project Title: Translational studies on ligand modified chitosan nanoparticles, A targeted nonviral gene transfer technology for enhancing the immunogenicity of intranasal DNA vaccines

Dates of Project: September 1, 2002 – August 31, 2003

Funded budget: \$97,000

Goal: Biomaterial nanoparticles for DNA vaccines and gene therapy (Targeted delivery of DNA vaccines using chitosan nanoparticles encapsulating DNA for induction of mucosal immunity)

GTEC Research Grant: National Science Foundation Georgia Tech/Emory Center for the Engineering of Living Tissues

Project Title: Mechanisms of Immune Responses Towards Antigens from Tissue Engineered Devices

Dates of Project: September 1, 2002 – August 31, 2003

Funded budget: \$79,567

Goal: Adjuvanticity of biomaterials in tissue engineering in the enhancement of immune responses to associated antigens (Antibody generation and T cell responses to a model antigen delivered with biomaterial scaffolds, microparticles and encapsulated cells)

NSF CAREER Award: National Science Foundation

Project Title: CAREER: Innate and Adaptive Immunity in Tissue Engineering

Dates of Project: July 1, 2003 – June 30, 2008

Funded Budget: \$400,000

Ranked 1st in study section

Goal: Assess the extent and mechanism of dendritic cell maturation upon biomaterial contact, and the biomaterial associated molecular controls.

GTEC Research Grant: National Science Foundation Georgia Tech/Emory Center for the Engineering of Living Tissues

Project Title: Mechanisms of Immune Responses Towards Antigens from Tissue Engineered Devices

Dates of Project: September 1, 2003 – August 31, 2004

Funded budget: \$80,686

Goal: Adjuvanticity of biomaterials in tissue engineering in the enhancement of immune responses to associated antigens (Antibody generation and T cell responses to a model antigen delivered with biomaterial scaffolds, microparticles and encapsulated cells)

EmTech Bio Seed Grant: EmTech Biotechnology Development, Inc.

Co-investigator: Ifor Williams, MD/PhD, Emory University

Project Title: A Targeted Delivery Technology for Enhancing the Immunogenicity of Intranasal DNA Vaccines

Expected Dates of Project: July 1, 2004 – June 30, 2005

Expected budget: \$100,000 (submitted but not funded)

Goal: Biomaterial nanoparticles for DNA vaccines and gene therapy (Targeted delivery of DNA vaccines for induction of mucosal immunity)

CDC/GIT Seed Grant: CDC/GIT Collaborative Research Program

Co-investigator: Jacqueline Katz, PhD, Centers for Disease Control and Prevention

Project Title: Enhancing the immunogenicity of avian influenza vaccines by modulating innate immunity with novel microparticles

Dates of Project: July 1, 2004 – June 30, 2006

Funded budget: \$60,000

Goal: Biomaterial microparticles complexed with toll-like receptor ligands for enhanced immune responses to avian influenza vaccines

GTEC Research Grant: National Science Foundation Georgia Tech/Emory Center for the Engineering of Living Tissues

Project Title: Mechanisms of Immune Responses Towards Antigens from Tissue Engineered Devices

Dates of Project: September 1, 2004 – August 31, 2005

Funded budget: \$85,732

Goal: Mechanisms of immune responses towards antigens from tissue engineered devices (danger signals associated with biomaterial implant site and effects on immune responses)

Whitaker Biomedical Engineering Research Grants Transitional Funding: The Whitaker Foundation

Project Title: Functional Consequences of Activated Leukocytes in Tissue Engineering

Dates of Project: September 1, 2005 – August 31, 2006

Funded budget: \$80,000

Goal: Assess the role of biomaterial activated leukocytes in the cross talk between endothelial cells and smooth muscle cells in the context of in stent restenosis and vascular tissue engineering

National Institutes of Health 1RO1EB004633-01A1

Project Title: Dendritic Cell Phenotype Upon Contact With Biomaterials

Dates of Project: August 1, 2005 – May 31, 2009

Funded budget: \$1,210,140

Goal: Assess the mechanism of dendritic cell maturation upon biomaterial contact and in vivo implications in tissue engineering.

GTEC Research Grant: National Science Foundation Georgia Tech/Emory Center for the Engineering of Living Tissues

Project Title: Mechanisms of Immune Responses Towards Antigens from Tissue Engineered Devices

Dates of Project: September 1, 2005 – August 31, 2006

Funded budget: \$80,032

Goal: Mechanisms of immune responses towards antigens from tissue engineered devices (danger signals associated with biomaterial implant site and effects on immune responses)

GTEC Research Grant: National Science Foundation Georgia Tech/Emory Center for the Engineering of Living Tissues

Project Title: Mechanisms of Immune Responses Towards Antigens from Tissue Engineered Devices

Dates of Project: September 1, 2006 – August 31, 2007

Funded budget: \$84,688

Goal: Mechanisms of immune responses towards antigens from tissue engineered devices (danger signals associated with biomaterial implant site and effects on immune responses)